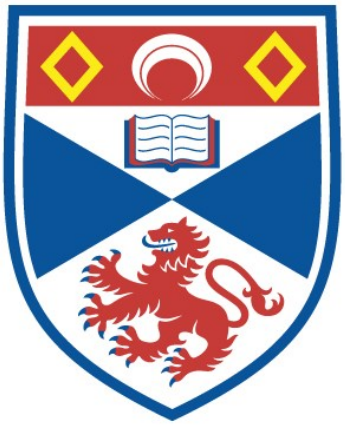
THE MINOR ALKALOIDS OF IPECACUANHA

George Cameron Davidson

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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The Minor Alkaloids of Ipecacuanha

being a Thesis

presented for the Degree of Doctor of Philosophy

of

the University of St.Andrews

by

George Cameron Davidson, B.Sc..

Declaration

I hereby declare that the following thesis is a record of the results of experiments carried out by me, that the thesis is of my own composition, and that it has not previously been presented for a higher degree.

The research was carried out in the Chemical Research Laboratory of the United College of St.Leonards and St.Salvator, St.Andrews University, and in the Department of Organic Chemistry, the University of Bristol, under the direction of Dr. A.R. Battersby, B.Sc., M.Sc.

University & Research Training

I attended the University of St.Andrews from October 1948 to June 1952, studying for the degree of B.Sc., and graduated with 2nd class Honours in Chemistry in July 1952. I studied for the degree of Doctor of Philosophy in the Chemical Laboratories, United College, St.Andrews from October, 1952 until January, 1954 and thereafter in the Department of Organic Chemistry at Bristol University until May, 1955. During the entire period of research the work was directed by Dr. A.R. Battersby.

Acknowledgments

I wish to acknowledge most sincerely the help and encouragement given to me at all times by Dr. A.R. Battersby.

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INTRODUCTION

Ipecacuanha had been used for many years in medical practice before Rogers (1), in 1912, showed that emetine, one of the constituent alkaloids, was a specific agent against Entamoeba Histolytica which is responsible for amoebic dysentery. This discovery, in relation to the toll which the disease takes in tropical countries, intensified the efforts of chemists to deduce the structure of the alkaloid in the hope of synthesising it. This hope was not finally achieved until 1950.

The final stages in selecting the correct structure for emetine were aided by a consideration of the possible biogenetic route to the alkaloid in the light of current theories of the biogenesis of alkaloids. In consequence of this, a detailed examination of the alkaloidal material present in the root was undertaken in the hope of isolating some compound which might be a biogenetic intermediate. The results of that investigation are described in this thesis. At the same time the opportunity was taken to study the chemistry of the minor alkaloids.

The work on the structural formula of emetine had shown the marked specificity of the alkaloid in the treatment of amoebic dysentery. There are four centres of assymetry in the molecule and inversion of the configuration at one is sufficient to destroy the therapeutic activity. Before a stereospecific synthesis of the alkaloid could be attempted the relative, and preferably the absolute, configurations at the asymmetric centres had to be ascertained. In the latter part of the thesis the initial experiments in the elucidation of the stereochemistry of emetine are described.

These results and the subsequent elucidation of the stereochemistry and the stereospecific synthesis of emetine and emetamine have been reported by Dr. Battersby and his associates in a series of papers in the Journal of the Society of Chemistry and Industry and the Journal of the Chemical Society (80)(56)(57).

The Biogenesis of Alkaloids

The complex nature of the alkaloids led to speculation as to how they might be elaborated from simpler units in the plant. The resultant hypotheses have been of great value both in structural determination and in synthesis. However, these purely organic hypotheses have tended to be structural rather than sequential in nature. They have indicated a structural relationship between alkaloids which supported the concept of their development from common precursors by various mechanistic paths rather than indicating the sequence of these changes and the identity of the intermediates. The guiding principles which have governed the development of biogenetic theories have been the existence of a mechanism whereby the postulated intermediates can interact as required and the adaptability of that mechanism to plant conditions.

Evidence in support of these theories has been sought by attempts to synthesise alkaloids under physiological conditions, i.e., by allowing reactants which could readily be derived from known naturally occurring substances to interact under conditions which parallel

those likely to exist in the living cell. A large number of these syntheses have been effected and a considerable number have shown stereospecificity in favour of the naturally occurring compound.

Despite the fact that these syntheses were never intended to be more than "laboratory summaries of the complex operations which the plant performs" and that neither the exact sequence in which the plant carried out the operations nor the exact nature of the intermediates has ever been postulated, the theory has been severely criticised by Dawson[2]. The arguments have been summarised by Hughes and Ritchie[3] as follows.

Against the theory it has been said that

(a) the postulated reactants have not been proved to exist in the plant cell;

(b) the plant synthesis is undoubtedly a complex series of enzymatic reactions;

(c) no definite positive evidence for the simple reactions postulated can be adduced.

In reply to this the following arguments have been propounded:

(a) such a large number of alkaloids of diverse types have had their molecular structure successfully

analysed that the possibility of mere coincidence is excluded;

(b) many alkaloids occur in optically active modifications and only in some cases have non-enzymatic reactions been proposed;

(c) as the proposed reactants always occur in low concentration and have a short life period due to their high reactivity, it was not to be expected that they would be detectable by techniques prior to those recently introduced.

In recent years with the advent of radio-isotopes and the development of mutant strains of plants it has been possible to test the hypotheses by the feeding of labelled intermediates and to begin the study of the sequential nature of the reactions.

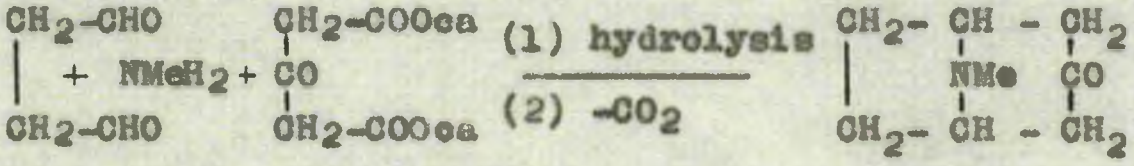
The evidence now available allows complete acceptance of the theories of biogenesis and leaves only the detailed nature of the reactions to be elaborated by the biochemist.

The extensive literature on the biogenesis of alkaloids has been the subject of several reviews[3][4] and this account will be limited primarily to the isoquinoline alkaloids.

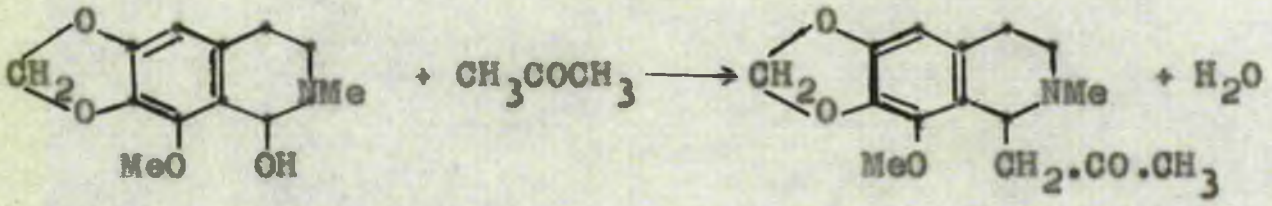
Amino acids and carbohydrates were early accepted as the most probable starting materials for the production of alkaloids in the plants. β-phenylethylamine and its derivatives, the source of which is undoubtedly phenylalanine and its nuclear substituted derivatives, are the most likely precursors of the isoquinoline system. Ring closure of these compounds to an isoquinoline can be brought about by condensation with an aldehyde.

To explain the formation of the papaverine series Winterstein and Trier[5] proposed that 3:4 dihydroxy-β-phenylethylamine(V) condensed with 3:4 dihydroxyphenylacetaldehyde(VI) to form a tetrahydroisoquinoline which by subsequent oxidation and methylation could yield papaverine. The former reactant could be derived from 3:4 dihydroxyphenylalanine by decarboxylation whilst the aldehyde could be derived from the same precursor by deamination to a substituted phenylpyruvic acid which subsequently decarboxylated. Such a scheme allowed the synthesis of papaverine from a known amino acid. However, the accepted methods by which these transformations could be effected in the laboratory were much more vigorous than those available to the plant.

In 1917 Robinson[6] reported the first synthesis of an alkaloid under conditions which simulated those available to the plant and employing reagents which were known to be present in nature. By allowing glutaraldehyde, methylamine and calcium acetone dicarboxylate to react in dilute aqueous solution at room temperature for 50 hours a base was obtained which on hydrolysis and decarboxylation gave tropinone (I) identical with the degradation product from tropine.



On the basis of this result, Robinson put forward his theory of the mechanism of the phytochemical synthesis of certain alkaloids[7], which has been the foundation upon which the structural relationships of natural products have been developed. In this theory the linking of carbon to carbon was held to occur by two processes only - the aldol condensation and the related condensation of carbinolamines, C(OH)N , with compounds containing the linkage, CHCO , as exemplified by the condensation of cotarnine (II) with acetone to give anhydrocotarnine acetone (III).



Cotarnine II Anhydrocotarnine III

$CH_3COCH(OH)CHO$

IV Acetylglycollaldehyde

The functional groups which it was necessary to postulate in order to give rise to the alkaloids were then ammonia, formaldehyde, ornithine, arginine and lysine together with degradation products of the carbohydrates. Acetone could be derived from citric acid in the form of acetone dicarboxylic acid. The quinoline and iso-quinoline alkaloids required the intervention of acetyl-glycollaldehyde (IV), a substance which, at that time, had not been isolated from natural sources but which could be derived from a methyl-pentose. The further steps envisaged were gentle oxidation, reduction, methylation and dehydration.

The theory has been criticised in that it accorded to the plant enzyme systems with too great a specificity in oxidation and dehydration. It has been suggested that by allowing sufficient liberality in the modes of dehydration,

oxidation and reduction any desired structure could be achieved.

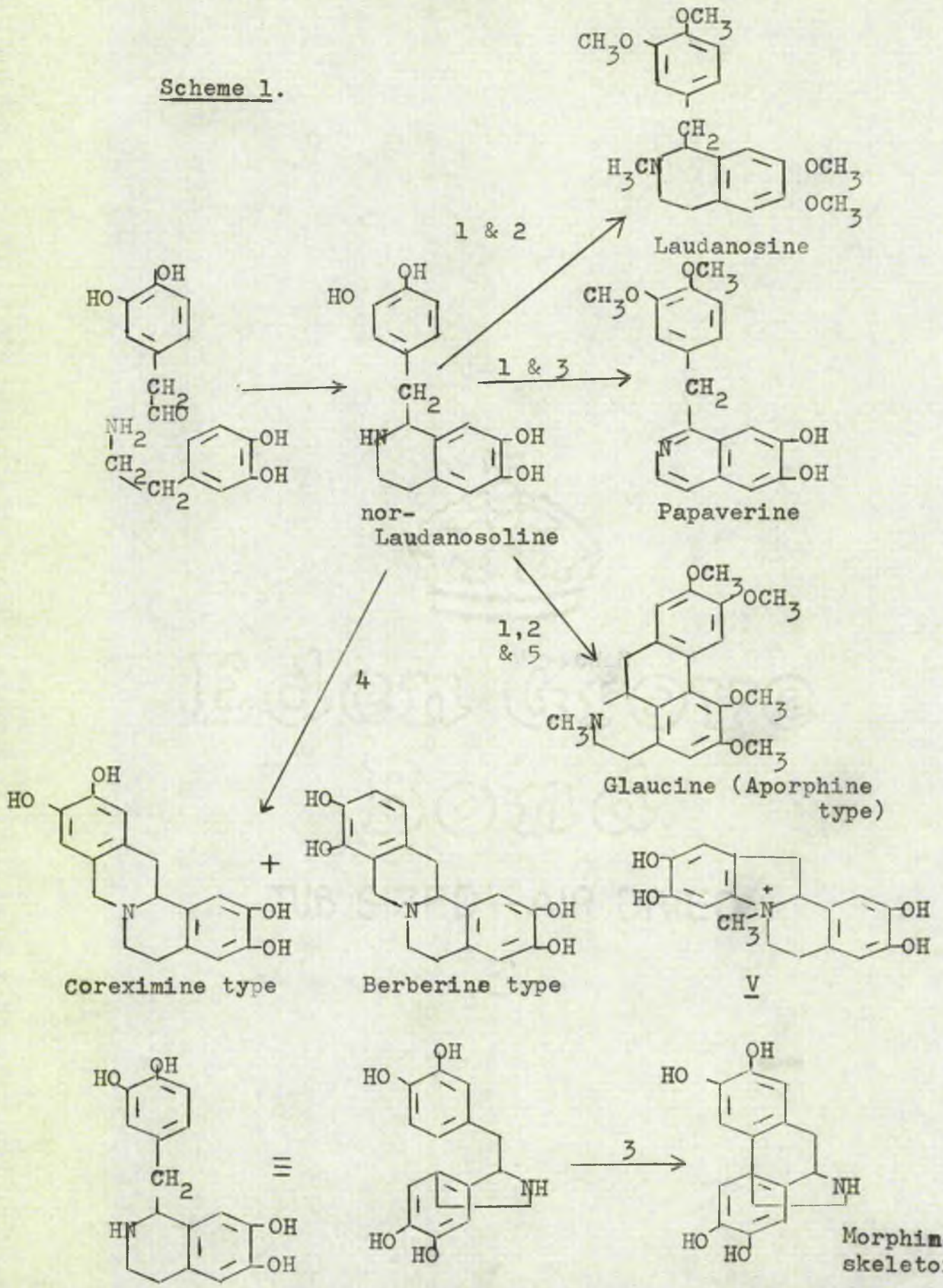
The chief contribution of Robinson's theory lies in the correlations which have been developed between alkaloids of apparently widely different structure.

The condensation of 3:4 dihydroxy-β-phenylethylamine and 3:4 dihydroxyphenylacetaldehyde leads to nor-laudanosoline which is held to be the precursor of all the isoquinoline alkaloids. Subsequent modification can lead to the representative papaverine, aporphine, protoberberine or morphine types as outlined in scheme 1(page 10).

The facility with which these reactions can take place has been demonstrated in the laboratory by Schöpf and Hahn and their associates in a large number of syntheses[12] under physiological conditions which were carried out under the inspiration of Robinson's synthesis of tropinone.

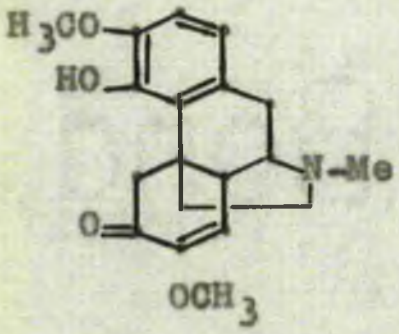
The attempts[8] to dehydrogenate nor-laudanosine to glaucine were unsuccessful, however, but led instead to a dehydrolaudanosoline-type quaternary alkaloid, V, a representative of which has since been found in nature[9].

Scheme 1.

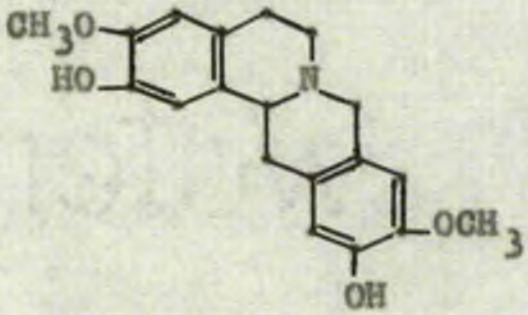


1 O-methylation
2 N-methylation
3 oxidation ($-H_2$)
4 formaldehyde condensation
5 chloranil dehydrogenation

Because of the distribution of the oxygenated functions in morphine itself, a biogenetic scheme based on tyrosine, which is now recognised as the intermediate in the conversion of phenylalanine to 3:4 dihydroxyphenylalanine, has been preferred for the morphine-thebaine group[10]. However, the alkaloid simoneine, VI, which possesses the morphine carbon skeleton would appear to be derived from 3:4 dihydroxyphenylalanine.



Simoneine, VI



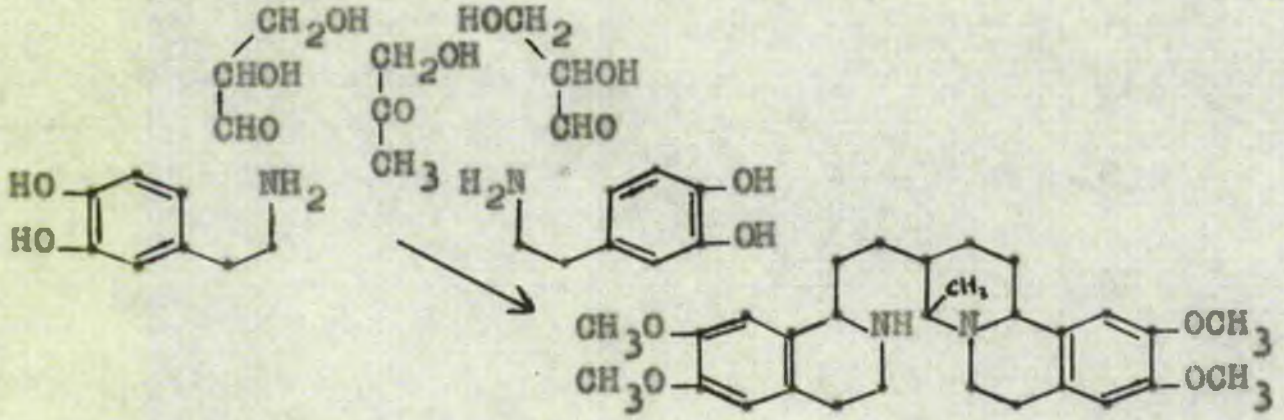
Coreximine, VII

The condensation of formaldehyde with nor-laudanosoline occurs readily but in the plant normally ortho to the hydroxyl function whereas in the laboratory the para condensation product is obtained. There is evidence of the natural occurrence of the para condensation in the protoberberine series with the isolation of the alkaloid Coreximine VII[11].

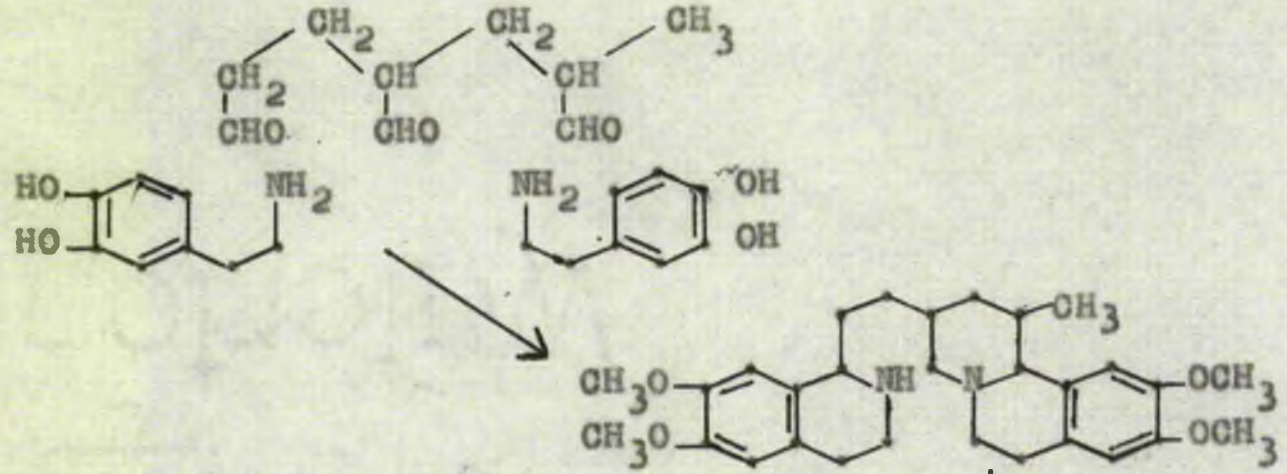
The application of the theory of biogenesis to structural problems has been extremely fruitful and was

invaluable in unravelling the structure of the morphine group. One of the first applications was to the problem of the structure of emetine. In 1925 emetine was known to have the formula $C_{29}H_{40}O_4N_2$, to contain four methoxyl groups, to be a secondary tertiary base, to contain one C-methyl group and to be a 1-substituted isoquinoline derivative. Furthermore, on oxidation with mild oxidising agents it was converted with the loss of eight hydrogen atoms to a deep red compound, rubremetine, in which one nitrogen had lost its basicity and the other was quaternary in nature. Robinson suggested that an alkaloid according with those properties could be derived from the condensation of two molecules of dihydroxyphenylalanine with two molecules of glyceraldehyde and one molecule of dihydroxyacetone with subsequent decarboxylation and reduction. In publishing this formula, VIII, Brindley and Pyman[13] commented on the inability of it to explain the character of rubremetine. They postulated structure IX in which the methyl group has been transposed and occupies a position related to that of the methyl group in corydaline. This formula requires the condensation of two molecules of dihydroxyphenylalanine with three molecules of glyceraldehyde. Rubremetine can then be

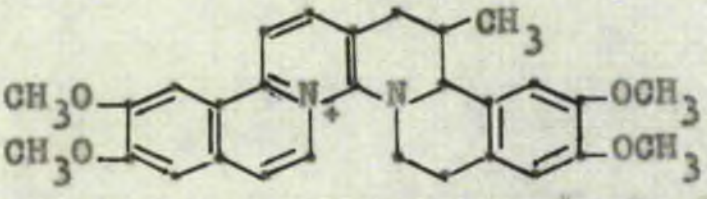
assigned formula X in which the colour and nature of the nitrogen functions is due to the existence of an amidine linkage.



VIII



IX



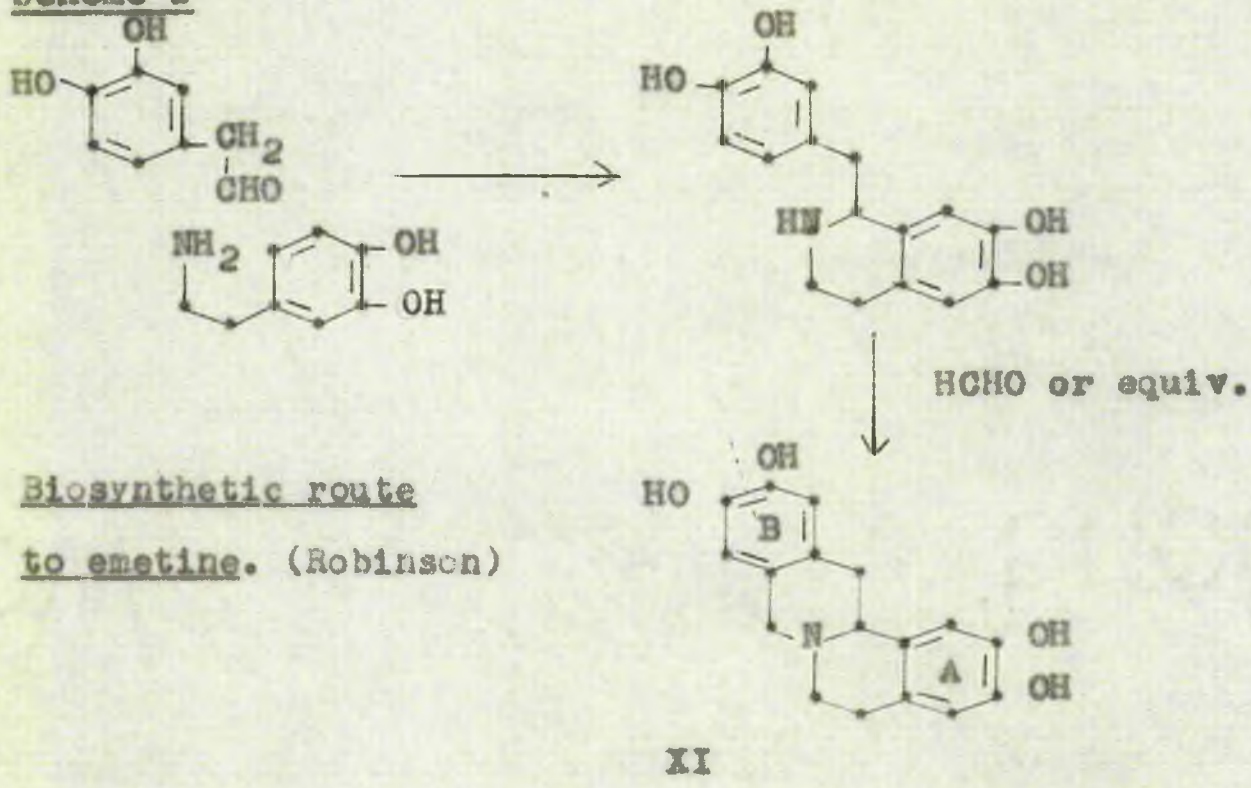
X

The structure IX was preferred to the alternative structure with the methyl group at C_9 on the basis of the nature of the reduction products of o-methylpsychotrine, a minor alkaloid which was believed to have a double bond at C_1-C_9. Reduction introduced a new centre of asymmetry at C_1 and two products were isolated, emetine and isoemetine. However, if the methyl group had been at C_9 two new centres of asymmetry would have been introduced and four diastereoisomers obtained.

As structure IX agreed with the partial formula suggested by Späth and Leithe[14] at the same time, it was accepted for many years until shown incorrect on the basis of Hofmann degradations of the alkaloid.

The accepted formula for emetine is now XII and the biosynthetic scheme which led Robinson[15] to propose this structure in 1948 is outlined in scheme 2. This structure was in agreement with the degradative evidence which had been published by Späth and Pailer[16] earlier in 1948 and was substantiated by the investigations of Pailer and Porschinski[73] and Battersby and Openshaw[74], the results of which were published in the following year.

Scheme 2



Biosynthetic route to emetine. (Robinson)

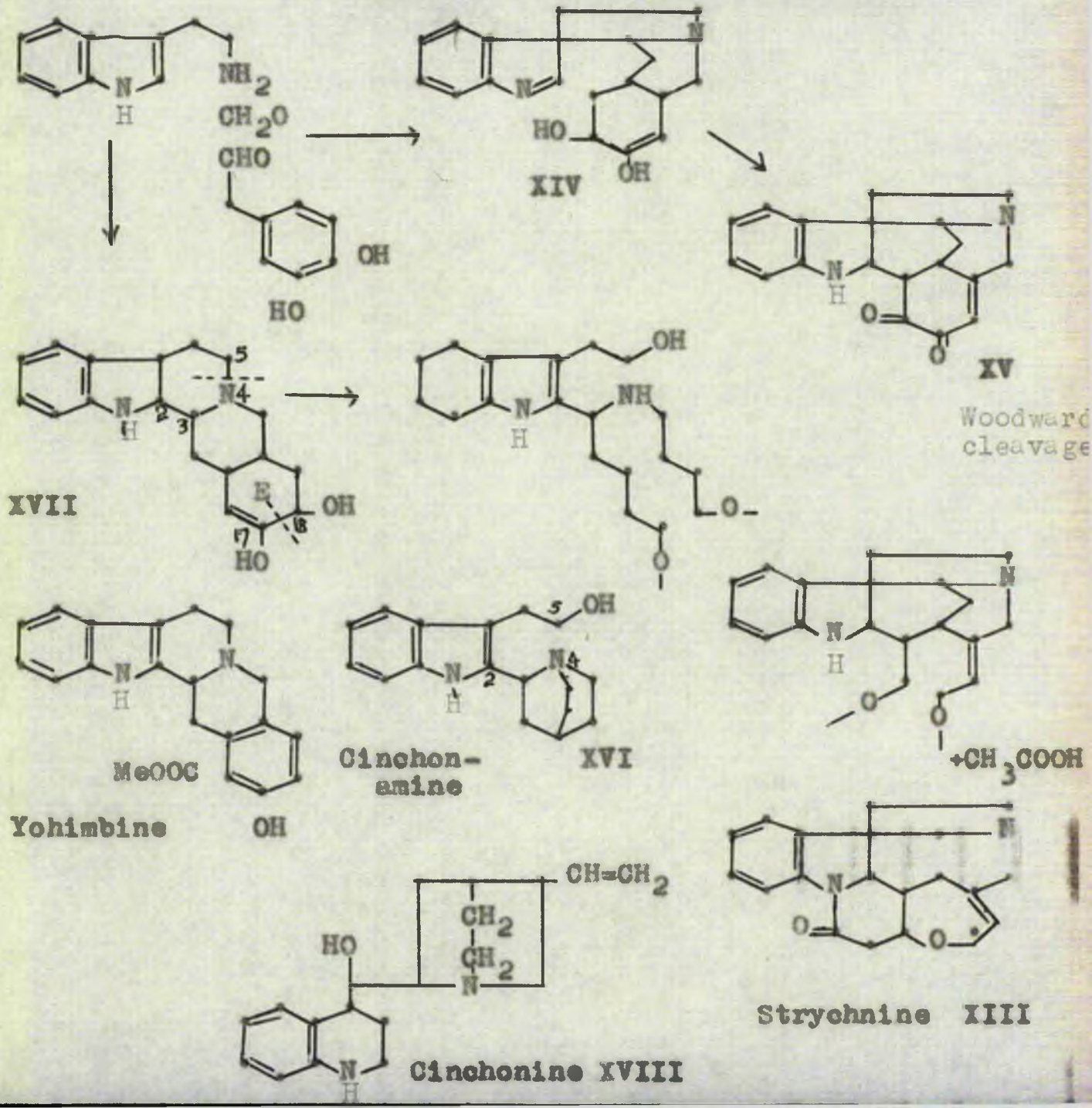
Emetine XII

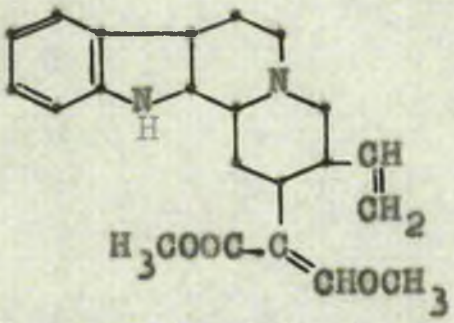
The first two steps in this scheme fit into the general pattern as outlined in scheme 2 but the third stage which invokes a fission of the B nucleus in the protoberberine, XI, marks the first application of a postulate made by Woodward to explain the biogenesis of strychnine XIII (17). This concept can only be substantiated by the degree of coincidence which has allowed the correlation of the structures of the Strychnos, Yohimbe and Cinchona alkaloids by minor modifications of a single biogenetic scheme based on tryptophan and phenylalanine and by the way in which it satisfactorily predicted the formula for emetine. It is now accepted as being as much an integral part of the theory of biogenesis as the processes of methylation, formylation, oxidation and reduction were of the original theory.

The ring fission of XI results in the formation of an unsaturated alcohol group, which undergoes reduction to form an ethyl group, and an aldehyde function which condenses with a further molecule of dihydroxyphenylalanine to yield on methylation etc. emetine. The exact order in which the reductive and methylating processes occur could not be specified.

An examination of the current schemes of biogenesis (4(a), 18) in the indole series, as outlined in scheme 3, emphasises the importance of the ring fission reaction in inter-relating apparently diverse groups.

Scheme 3. Biogenetic relationships between Yohimbine, Strychnine and Cinchona alkaloids.





Corynantheine XIX

The biogenetic scheme for yohimbine put forward by Hahn[19] depends upon the anionoid activity of the α-position in the indole nucleus to effect the initial ring closure between tryptamine derived from tryptophane and the aldehyde from dihydroxyphenylalanine. Woodward observed that if the condensation were to occur at the β-position in the nucleus which also can exhibit anionoid activity the resultant structure XIV bore a strong resemblance to that of strychnine. The formation of a new bond between the indolenine nucleus and the anionoid 2-position in the aromatic ring would be readily achieved but because of steric strain the bonds would probably rearrange to give structure XV. To obtain the seven-membered heterocyclic ring of strychnine it is necessary to postulate the fission of this diketone with one oxygenated function giving rise to the ether linkage

whilst the other condenses with an N-acetyl group. Thus the relationship between yohimbine and strychnine was established.

Among the minor alkaloids of cinchona, cinchonamine was shown to have the structure XVI containing an indole nucleus unlike the quinoline nucleus present in the other alkaloids of the series, e.g. cinchonine. A scheme for the interconversion of these two basic units was put forward by Janot, Prelez, Goutarel and Taylor[20] who noted that rupture of the N_1-C_2 bond in cinchonamine followed by a union of the N_1-C_5 atoms would give rise to the complete skeleton of the cinchona alkaloids.

Meanwhile a suggestion was put forward for the development of cinchonamine from XVII, the postulated precursor of the yohimbine series. In this, fission of the N_4-C_5 bond as previously observed in the berberine and benzophenanthridine groups was accompanied by a Woodward-type fission of the C_{17}-C_{18} linkage in the dihydroxy aromatic system. The formation of a new bond between C_{17} and N_4 led to the skeleton of cinchonamine.

Further evidence in favour of the fission of dihydroxy-aromatic systems was found when the structure of corynantheine was established as XIX. The side chains in this alkaloid are obviously derived from ring E of a

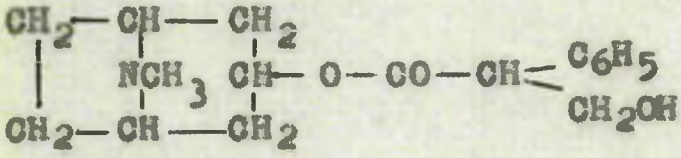
yohimbine-type of structure and add to the chain of circumstantial evidence in favour of this form of fission.

Until recently no direct proof existed of the correctness of the hypotheses which go to formulate the theory of biogenesis but the examination of the biosynthetic processes has now been pursued so far as to allow detailed knowledge of the individual steps in some of the simpler stages. Methionine, for instance, has been shown to be the source of the N-methyl group in the alkaloids of barley[21].

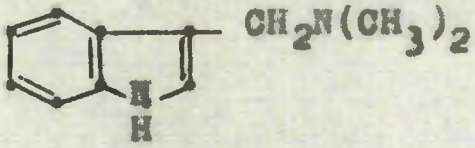
There were three obvious ways in which the problem could be broached - labelled precursors could be fed to the plants and the positions in which the labelled entities occurred in the alkaloid determined; the synthetic process could be halted at an intermediate stage and the intermediate product in the biogenesis isolated; finally, an intensive search could be made for intermediates which would normally be present in the plant in very low concentration dus to their high reactivity but which might be isolable with modern techniques. Each method has received attention. With the ready availability of C^{14}, the radio active isotope of carbon, the first route has received considerable attention and in the

alkaloid field has been pursued mainly by Marion and his co-workers in Canada.

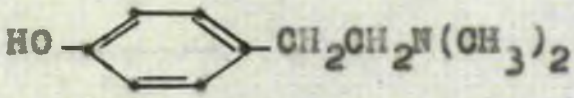
Some work has been undertaken by biologists on the effect of adding the postulated amino acid precursors on the final yield of alkaloid in the plant but the results were inconclusive[22]. Restricting themselves to the alkaloids of belladonna and barley the Canadian workers have fed labelled amino acids to the growing and mature plants. The alkaloids were then extracted and degraded to find where the radio activity was sited. In this way, ornithine has been shown to be the precursor of hyoscyamine(XX)[23], gramine (XXI) to be derived from tryptophan[24] and horsenine (XXII) from phenylalanine via tyrosine[25].



Hyoscyamine XX



Gramine XXI



Hordenine XXII

The procedure whereby the synthetic process is halted before completion is dependent on the development of mutant strains by the botanist. There are no instances of this having been achieved in the alkaloid field but it has given satisfying results in the study of the biosynthesis of the aromatic ring. This study, however, has also achieved its greatest impetus from the introduction of radioactive isotopes. The early proposals of Collie[26] based on his work on polyketens (dehydracetic acids), that acetic acid is the fundamental unit of synthesis have been substantiated. Finally, the course of the biosynthesis of glucose and therefrom phenylalanine via shikamic acid has been clearly demonstrated. This work has been excellently reviewed[27].

Finally, although in many instances closely related alkaloids have been isolated from the same plant, there appeared to have been no exhaustive search made for alkaloids which occurred in trace quantities and which might indicate the biosynthetic route. The results of such an investigation undertaken in the ipecacuanha series are recorded later in this thesis.

Isolation and Structure of the Alkaloids of Ipecacuanha

Cephaelis Ipecacuanha had been shown to contain five alkaloids whose structures are now established. Emetine, o-methylpsychotrine and emetamine are non-phenolic alkaloids, whereas cephaeline and psychotrine are phenolic in character. Besides these five bases, Hesse[28] has claimed to have isolated two further alkaloids, ipecamine and hydroipecamine, which were amorphous and formed amorphous salts. Merck[29] has also reported that alkaloids other than the original five are present. The extent to which the known alkaloids occur in the plant is recorded in Table 1.

Table 1

Alkaloid	wt.% dry root
Emetine	1.5 - 1.7
o-Methyl-psychotrine	0.015-0.033
Cephaeline	0.6 - 0.7
Psychotrine	0.04 - 0.06
Emetamine	0.002-0.006

Isolation of the alkaloids

The alkaloids are normally extracted from the

pulverised bark and roots along with a considerable amount of other material by ethanol at 70°C or methanol at 60°C after which they are extracted into an organic solvent which is normally ether, di-isopropyl ether or chloroform. The phenolic material is then removed by treatment with aqueous potassium hydroxide and the non-phenolic bases are subsequently extracted into dilute sulphuric acid.On the addition of bromide or iodide ions, the sparingly soluble emetine salt is precipitated from the acid extract. In one commercial process, the alkaloids remaining in the mother liquors are precipitated as a bismuth iodide complex.

Paul and Cownley[30] were the first to isolate pure emetine in the above manner and at the same time they isolated the two alkaloids from the phenolic fraction. Cephaeline was obtained as the hydrochloride from acid solution and psychotrine crystallised from an ethereal solution of the bases recovered from the mother liquors.

Pyman[31] in 1917 obtained o-methyl psychotrine and emetamine from the mother liquors of emetine hydrobromide. These two alkaloids form very sparingly soluble hydrogen oxalates and as such they were isolated. The separation of the two bases is best achieved by utilising the

difference in basicity between them whereby o-methylpsychotrine is preferentially extracted into dilute acid from a chloroform solution of the bases. Karrer (32) has utilised the reactivity of o-methylpsychotrine with succinic anhydride to achieve the separation. The half-amide so formed is soluble in cold alkali and readily saponified by hot alkali.

Pyman considered that neither ipecamine nor hydroipecamine were pure compounds since under the same conditions as these two bases were obtained both o-methylpsychotrine hydrobromide and emetamine hydrobromide would have been precipitated. However, these two bases are dextra rotatory whereas Hesse's bases were strongly laevo rotatory. Hence it must be presumed that there is present at least one strongly laevo-rotatory base which has not yet been isolated.

The Inter-relationship of the alkaloids

Although the structure of the emetine molecule was not elucidated until 1948, the inter-relationships of the alkaloids had been clearly established by Pyman and his co-workers [13][31][33] in their early researches.

Emetine, o-methylpsychotrine and emetamine were known to be non-phenolic in character whereas cephaeline and psychotrine were phenolic. From the methylation of cephaeline with methyl sulphate in sodium amyloxide solution emetine was obtained but if sodium methoxide was the base employed a mixture of N-methyl cephaeline, N-methyl emetine and only a little emetine resulted. The reverse reaction did not occur to give a preferential hydrolysis of one methoxyl group as was observed with o-methylpsychotrine which as the name indicates was obtained by the methylation of psychotrine.

Reduction of o-methylpsychotrine with sodium in alcohol led to a mixture of bases. One was isolated as its hydrobromide and shown to be emetine and another formed an N-acetyl derivative. Analyses showed the latter to be isomeric with emetine but the two alkaloids were not interconverted when treated with sodium amyloxide. However, each could be oxidised by iodine to

o-methylpsychotrine. It was presumed that reduction of the double bond introduced a new asymmetric centre and that the two bases, emetine and iso-emetine, were stereoisomers. From the reduction a third compound was isolated in which the double bond had been reduced and one methoxyl group had been eliminated. Taken in conjunction with the ready hydrolysis of one methoxyl group this result gave a strong pointer to the position of the double bond in o-methylpsychotrine as it was known that easy hydrogenolysis of alkoxy groups occurred at the para position in styrene derivatives[34].

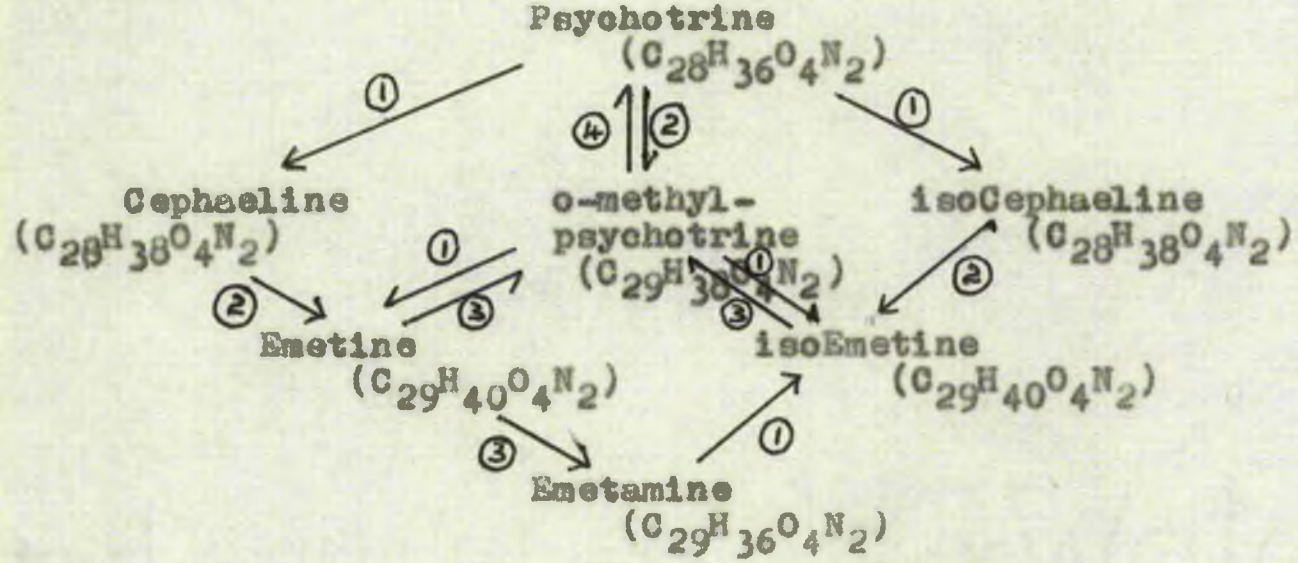
Similarly, the reduction of psychotrine with sodium and ethanol led to a mixture of cephaeline and isocephaeline which could in turn be methylated to emetine and iso-emetine.

Catalytic reduction of o-methylpsychotrine hydrogen oxalate in aqueous solution over a platinum catalyst, on the other hand, afforded isoemetine as the sole product[32].

That emetamine possessed the basic emetine skeleton was shown by the isolation of isoemetine when the alkaloid was reduced by the action of sodium in ethanol[13]. Confirmatory evidence was later obtained by Ahl and

Reichstein[36] who isolated emetamine in 25% yield when they submitted emetine to catalytic dehydrogenation over palladium charcoal.

These results can best be summarised diagrammatically as below. The interpretation of the results can then be discussed in relation to the accepted formula for emetine XXIII (R=Me)

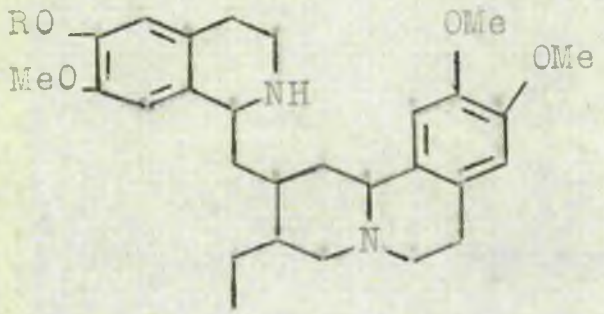


(1) reduction

(2) methylation

(3) oxidation (dehydrogenation)

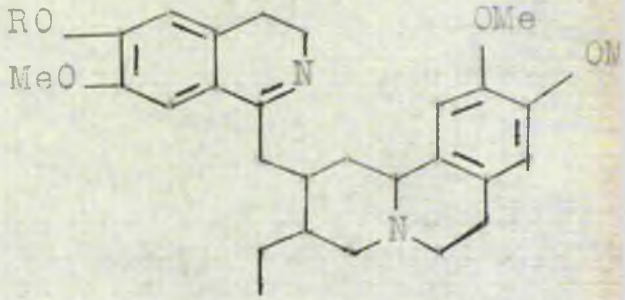
(4) acid hydrolysis



XXIII

Emetine (R=Me)

Cephaeline (R=H)



XXIV

O-Methylpsychotrine (R=Me)

psychotrine (R=H)

O-Methylpsychotrine was assigned the formula XXIV (R=Me). The double bond was ascribed to either position C_1-C_9 or C_1-N_2 on the basis of the extreme lability of one methoxyl group as has already been mentioned. As O-methylpsychotrine formed an N-benzoyl derivative Brindley and Pyman preferred the former position for the double bond. Karrer (32) concluded that the isolation of N-benzoyl corydaldine from this N-benzoyl derivative on either ozonolysis or oxidation with perphthalic acid was further evidence in favour of that structure.

However, the possibility does exist for tautomerism between -CH-C=N- and -C=C-NH- so that formation of the N-benzoyl derivative would result in a structure

with the exocyclic double bond[50]. That o-methyl-psychotrine did in fact possess an endocyclic double bond was concluded from a study of the ultra-violet absorption. Investigations into the ultra-violet absorption of 1-substituted dihydroisoquinolines undertaken by Bills and Noller[37] had shown that the endocyclic position for the double bond is favoured, even in the case of 1-benzyl 3:4 dihydroisoquinoline where the exocyclic double bond would be in conjunction with both benzene rings.

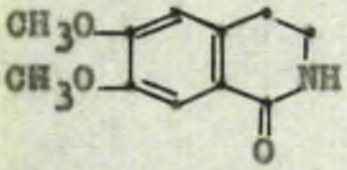
The reduction of o-methylpsychotrine introduces a new centre of asymmetry at C_1 which explained the relationship of emetine and iso-emetine and also of cephaeline and isocephaeline.

As the inter-relationship of emetine, cephaeline and psychotrine had been clearly established only the position of the phenolic hydroxy group in the former two remained to be settled. That this was correctly ascribed to C6 was proved when Späth and Leithe prepared o-ethyl cephaeline and oxidised it to a mixture of corydaldine (XXV) and 6-ethoxy-7-methoxy-1-keto-1,2,3,4 tetrahydroisoquinoline (XXVI). Confirmation was

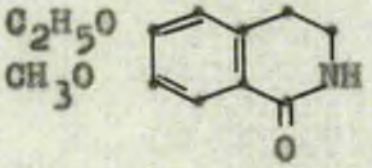
obtained when Pailer and Porschinski degraded o-ethyl cephaeline by a series of Hofmann degradations to a carbonyl compound which differed from that obtained from emetine by a similar degradation and from a synthetic compound with the ethoxy group in the 7-position. The structures of cephaeline and psychotrine were thus XXIII (R=H) and XXIV (R=H). There remained to be decided only the structure of emetamine.

Emetamine had been ascribed the empirical formula $C_{29}H_{36}O_4N_2$. It was a ditertiary base and a weaker base than o-methylpsychotrine. On oxidation with mild oxidising agents it gave a different red compound from o-methylpsychotrine and, as already stated, it was reduced to iso-emetine. Emetamine was ascribed formula XXVII in which ring B is fully aromatic. The evolution of nearly two moles of hydrogen in the dehydrogenation of emetine to isoemetine was adjudged as further evidence for this formula by Ahl and Reichstein. However, as 50% of the emetine was converted to 1-methyl-6,7-dimethoxy-isoquinoline the author is of the opinion that it was only fortuitous that the volume of hydrogen evolved was correct for a total conversion to emetamine. Although

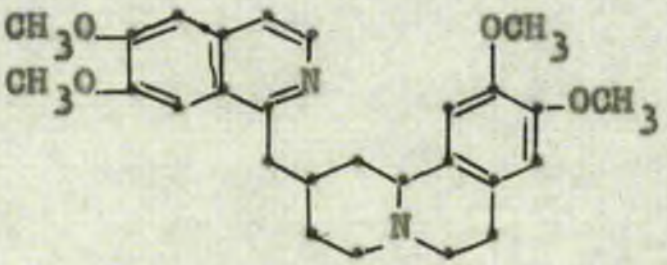
the chemistry of emetamine can be explained on the basis of formula XXVII there is no experimental evidence that emetamine does possess a fully aromatic isoquinoline system



Corydaldine XXV



XXVI



Emetamine XXVII

Oxidation of the Ipecacuanha alkaloids

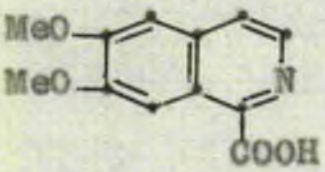
(1) Degradative oxidation

The structure of emetine has now been universally accepted as XXIII and the evidence leading to that acceptance has been adequately reviewed by Janot[38].

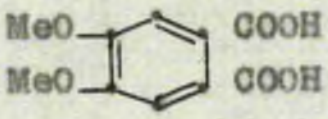
Degradative oxidation has played a major part in elucidating many complex structures. In the case of emetine and its related alkaloids this has not been so. Although helped by oxidative studies the structural complexity was unravelled only after extensive use of the Hofmann degradation. In no instance did oxidation of emetine lead to the isolation of large fragments of the molecule in good yield.

Early attempts to oxidise emetine with nitric acid led only to the isolation of oxalic acid[39] and later to nitrated compounds with a musk-like odour[40]. Pyman, to whom must be attributed the major credit in isolating and characterising the alkaloids of this group, studied the effect of potassium permanganate in aqueous acetone on emetine and isolated 6,7-dimethoxyisoquinoline-1-carboxylic acid (XXVIII) and m-Hemipinic acid (XXIX),

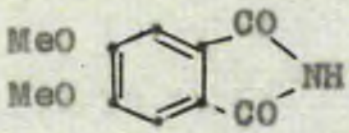
thus giving the first clue to the existence of an isoquinoline nucleus in the alkaloid. At about the same time, Windaus and Hermanns[41] oxidised emetine hydrochloride with aqueous permanganate when complete disruption of the molecule again occurred and the only products isolated were m-hemipinimide (XXX) and m-hemipinic acid.



XXVIII

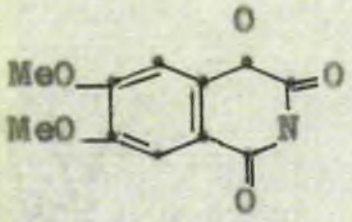


XXIX

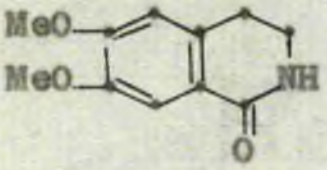


XXX

Hermanns[42] carried out the oxidation of emetine with chromic acid and isolated 4,5-dimethoxyphthalonimide (XXXI) which gave support to the presence of an isoquinoline nucleus. Consideration of the nature of this last oxidation product and the known fact that of the two nitrogen atoms in the molecule one was tertiary and the other secondary, suggested that the isoquinoline group was probably in the reduced state and that the aromatic isoquinoline system XXVIII had been generated in the oxidation.



XXXIII



XXXIV

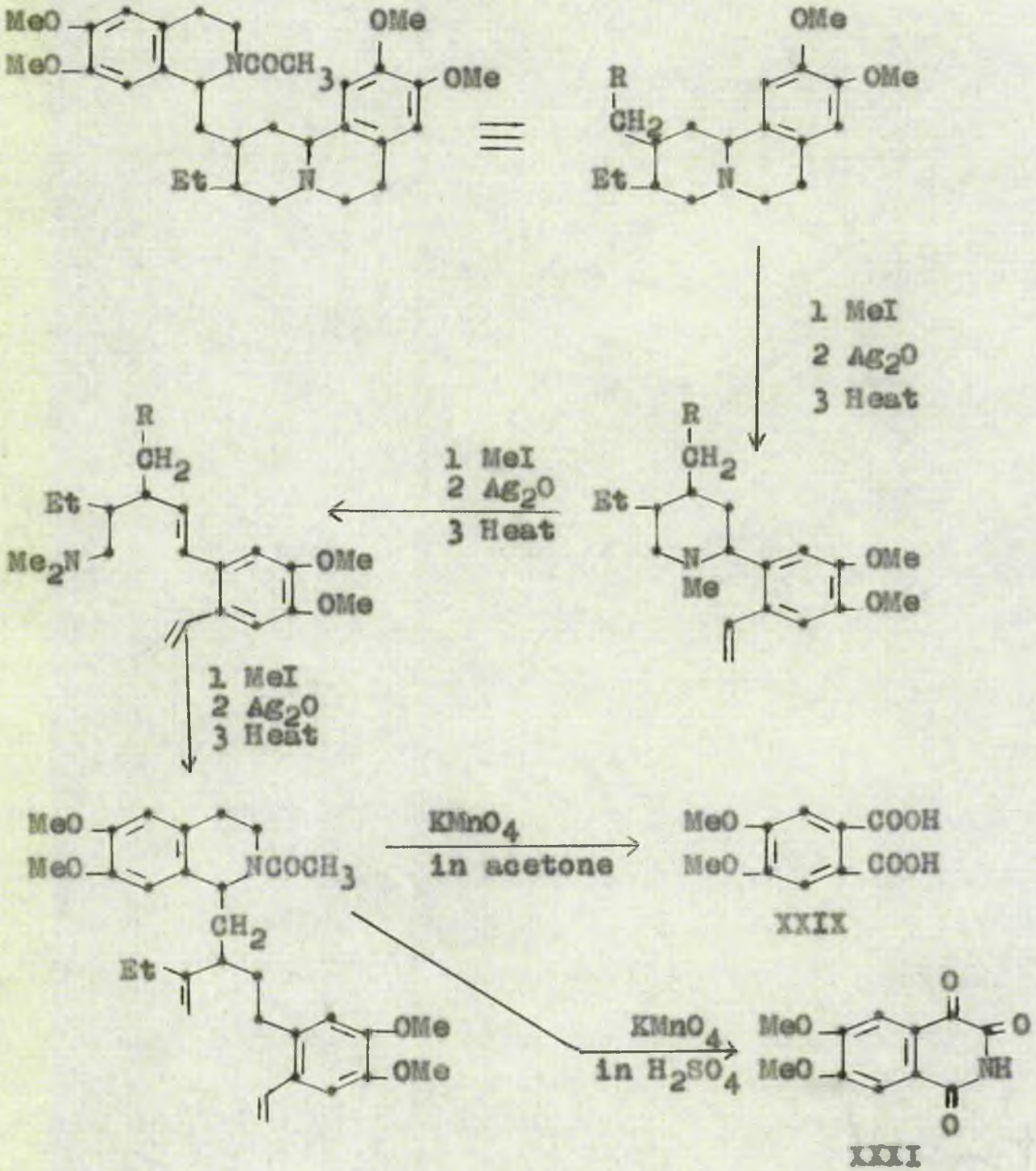
When a slightly alkaline solution of potassium permanganate was employed in the oxidation, corydaldine XXXII as well as m-hemipinic acid was isolated. On correcting for the corydaldine present the yield of m-hemipinic acid in this instance was 96% as compared with a normal yield of 35-40% when alkaloids containing one isoquinoline nucleus (e.g. papaverine) were oxidised under similar conditions. This result was taken as a firm indication of the presence of two isoquinoline nuclei.

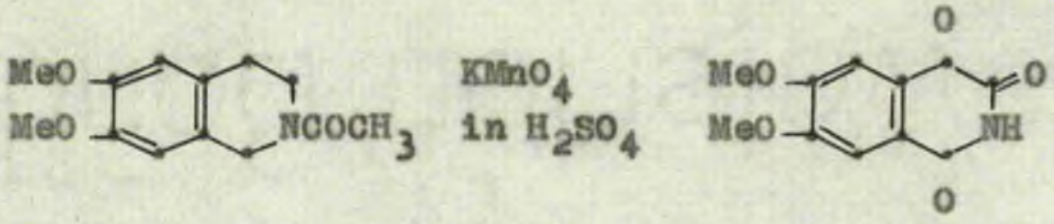
Oxidation of N-benzoylemetine in the hope of finding out which nucleus gave rise to corydaldine resulted only in the isolation of a poor yield of 3:4-dimethoxy-6-β-benzamido-ethyl benzoic acid (XXXIII). However, the oxidation of o-ethyl cephaeline to yield a mixture of corydaldine and 6-ethoxy-7-methoxy-1-keto-1,2,3,4-tetrahydroisoquinoline verified the presence of two tetrahydroisoquinoline nuclei in the molecule. The latter

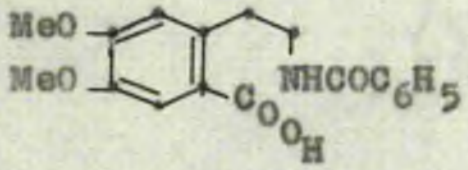
compound was further oxidised to 4-methoxy-5-ethoxy-phthalic acid.

The oxidation of o-methylpsychotrine with aqueous permanganate had given 6:7-dimethoxyisoquinoline-1-carboxylic acid and rubremetine hydrobromide (p. 4) on similar treatment had given m-hemipinimide and m-hemipinic acid as the only identifiable products(43). Two major products in which the carbon skeleton had not been disrupted were never identified.

The unambiguous assignment of the secondary nitrogen atom to a dimethoxy tetrahydroisoquinoline ring was achieved in 1944 when Ahl and Reichstein(36), on degrading N-acetyl emetine by successive Hofmann reactions, obtained a neutral compound which was oxidised by permanganate in dilute sulphuric acid to 4:5-dimethoxyphthalonimide, identical with that obtained earlier by Hermanns. Oxidation with permanganate in acetone, however, had only yielded m-hemipinic acid. The structure of the imide was confirmed when it was obtained by a similar oxidation of N-acetyl-6:7-dimethoxy 1,2,3,4-tetrahydro-isoquinoline or the free base. The sequence of reactions is outlined below assuming the correct structure for emetine.

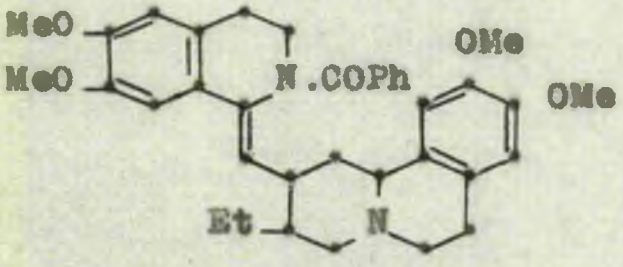

MeO
MeO
NCOCH3
OMe
OMe
Et
N
≡
R
CH2
1 MeI
2 Ag2O
3 Heat
Me2N
Me
KMnO4
in acetone
COOH
XXIX
KMnO4
in H2SO4
NH
O
XXII





XXXIII

N-benzoyl-o-methylpsychotrine (XXXIV) possesses the double bond in the exocyclic position and has been both oxidised with perphthalic acid and subjected to ozonolysis in an attempt to achieve a clean fission of the molecule but only trace quantities of N-benzoyl corydaldine were isolated.



XXXIV

N-benzoyl-o-methyl-
psychotrine

The only other oxidations in the emetine series have been on the products of exhaustive Hofmann degradative and are not true degradative oxidations of the bases.

It can thus be seen that in none of the oxidations carried out has any product in which the tricyclic system remains intact been isolated. In order to study the stereochemistry of the alkaloids it would be necessary to isolate such a compound. From this review a further investigation of the oxidation of N-benzoyl-o-methyl-psychotrine appeared to offer most promise.

(ii) Catalytic dehydrogenation

The dehydration of emetine over palladium charcoal at 180-190°C has been reported[36] to give rise to emetamine with the evolution of two moles of hydrogen whilst at the same time 1-methyl-6:7-dimethoxyisoquinoline was isolated. Wood[44], however, isolated as the major product of dehydrogenation o-methylpsychotrine together with 1-methyl-6:7-dimethoxyisoquinoline but no emetamine. As the product obtained by Ahl and Reichstein was identical in properties with naturally occurring emetamine there can be little doubt of its authenticity. Catalytic dehydrogenation of emetine or o-methylpsychotrine would be the easiest route to emetamine but all attempts to repeat the initial experiment have been unsuccessful.

(iii) Chemical dehydrogenation

Emetine is converted into a red quaternary compound upon treatment with mild oxidising agents in acid solution. The salts of this compound have been termed rubremetinium salts for which the generic term rubremetine will be employed hereafter.

Carr and Pyman[33a] obtained the deep red quaternary salt by oxidising emetine with ferric chloride. It was shown to have the formula $C_{29}H_{32}O_4N_2.HCl$ whence eight atoms of hydrogen had been abstracted from the emetine molecule whilst one nitrogen had lost its basicity and the other had become quaternary in nature. After having treated emetine with alcoholic iodine, Karrer[45] isolated a golden-yellow compound, dehydroemetine, which he agreed later[46] to be identical in properties with rubremetine. The author has always found it difficult to reconcile the colour difference between the two compounds. Pyman[31], however, confirmed that oxidation of emetine with four molecular proportions of iodine gave rise to rubremetine and showed that bromine could convert o-methylpsychotrine to the same compound. Iso-emetine was also converted to rubremetine by oxidation with

ferric chloride but N-methylemetine was not.

Emetamine yielded a similar red quaternary salt with bromine in chloroform[13] but from the melting point this was a different compound from rubremetine. This difference was confirmed by Karrer, Engster and Rüttner[32] who compared the ultra-violet absorption spectra of the two compounds.

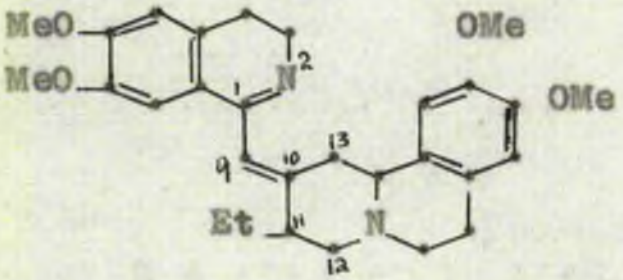
Some indication as to the course of the reaction was obtained when both emetine[31] and iso-emetine[33b] upon treatment with one molecular proportion of iodine gave rise to o-methylpsychotrine.

The use of mercuric acetate for the preparation of rubremetine was introduced by Battersby and Openshaw[47] who isolated a second intermediate, tetradehydroemetine, which was further oxidised with mercuric acetate to rubremetine.

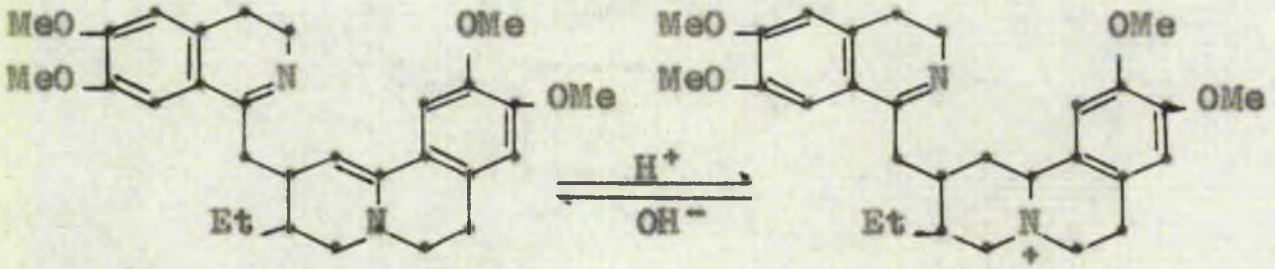
Structure of tetradehydroemetine

At the time the structure XXXV was proposed for this compound[47] as micro-hydrogenation indicated the presence of two double bonds and the ultra-violet absorption spectrum suggested that these were conjugated with one another and with an aromatic ring. This structure

was reconsidered in a later paper[48] in light of the work of Bills and Noller[37] and upon consideration of the relative stabilities of the salts and free base. Formula XXXVI was accepted which would rearrange to XXXVII in acid solution.



XXXV



XXXVI XXXVII

On repeating this work, Hazlett and McEwen[48] obtained the same compound but along with it another compound, isotetradehydroemetine, which possessed an almost identical ultra-violet absorption, was oxidised further to rubremetine and analysed for $C_{29}H_{36}O_4N_2$. When it was reduced catalytically over platinum, either

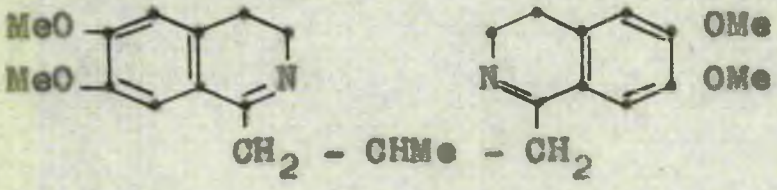
as the free base or a salt, 1.85 moles of hydrogen were absorbed but emetine in 37% yield was the only product isolated.

The reduction of tetradehydroemetine on the other hand took a different course dependent upon the conditions employed. On reduction of the hydrogen oxalate in aqueous solution two moles of hydrogen were absorbed and iso-emetine (78%), as its N-benzoyl derivative, was the only product isolated[48]. When an alcoholic suspension of the hydrogen oxalate was hydrogenated over a platinum catalyst two moles of hydrogen were again absorbed and from the reduction mixture were isolated emetine and iso-emetine along with two new compounds which were assumed to be stereoisomers of emetine and designated neoemetine and emetine IV.

Any structure for these isomeric tetradehydroemetines had to account for the similarity in their ultra violet absorptions which precluded any major difference in the distribution of the double bonds. Openshaw and Wood regarded tetradehydroemetine as XXXVI in view of the marked resemblance of the ultraviolet absorption to that of a model bis-3:4-dihydroisoquinoline, XXXVIII, and did not commit themselves to a structure for iso-tetrahydro-emetine.

Hazlett and McEwen suggest that the compounds are either geometrical isomers about a $C_1 - C_9$ double bond, XXXIX, or else that the second double bonds are located at $C_{10} - C_{11}$ and $C_{10} - C_{13}$, respectively, but they permit themselves no decision on the matter.

The possibility that isomerism around the $C_1 - C_9$ bond is responsible for the difference in physical properties can be ruled out as 3:4-dihydroisoquinolines are known to exist with endocyclic double bonds in acid solution and any isomerism would be destroyed in the salts. This is not the case. Thus, the exact nature of these compounds is still open to doubt.



XXXVIII

Reduction of rubremetine

The reduction of rubremetine has been characterised by the isolation of a diversity of products according to the conditions employed.

Karrer, Engster and Rüttner[32] reduced rubremetine with zinc and acetic acid and isolated a single product, tetrahydrodehydroemetine, $[\alpha]_D^{18} = 41.5^\circ$ (in ethanol),

m.p. 134°C. This material was resistant to catalytic hydrogenation.

Isolated from the reduction of rubremetine with lithium aluminium hydride[51] was a single compound which analysed as a dihydrodehydroemetine $C_{29}H_{34}O_4N_2$ m.p. 157-8°, $[\alpha]_D$ + 38° (in ethanol). On subsequent catalytic hydrogenation in acetic acid solution one mole of hydrogen was absorbed and two compounds were isolated. One of these was identical with tetrahydrodehydroemetine and the other had m.p. 194°, $[\alpha]_D$ -380° (in ethanol). McEwen and Tietz later confirmed the nature of the reduction products but reported that the hydrogenation involved the uptake of two moles of hydrogen.

Meanwhile, Openshaw and Wood[48] had repeated the earlier[47] catalytic reductions in sodium acetate buffered solution. A rapid absorption of one mole of hydrogen occurred and two crystalline reduction products were isolated, α-dihydrorubremetine, m.p. 198° $[\alpha]_D$ - 395° (in acetone) and β-dihydrorubremetine, m.p. 202° $[\alpha]_D$ + 406°(in acetone). From methanol these two compounds crystallised as a solvated complex, m.p. 128° $[\alpha]_D$ + 20° (in acetone). This behaviour suggested that the α- and β-dihydrorubremetines were diastereoisomers.

Both compounds were reoxidised to rubremetine and gave reactions characteristic of a pyrrole nucleus.

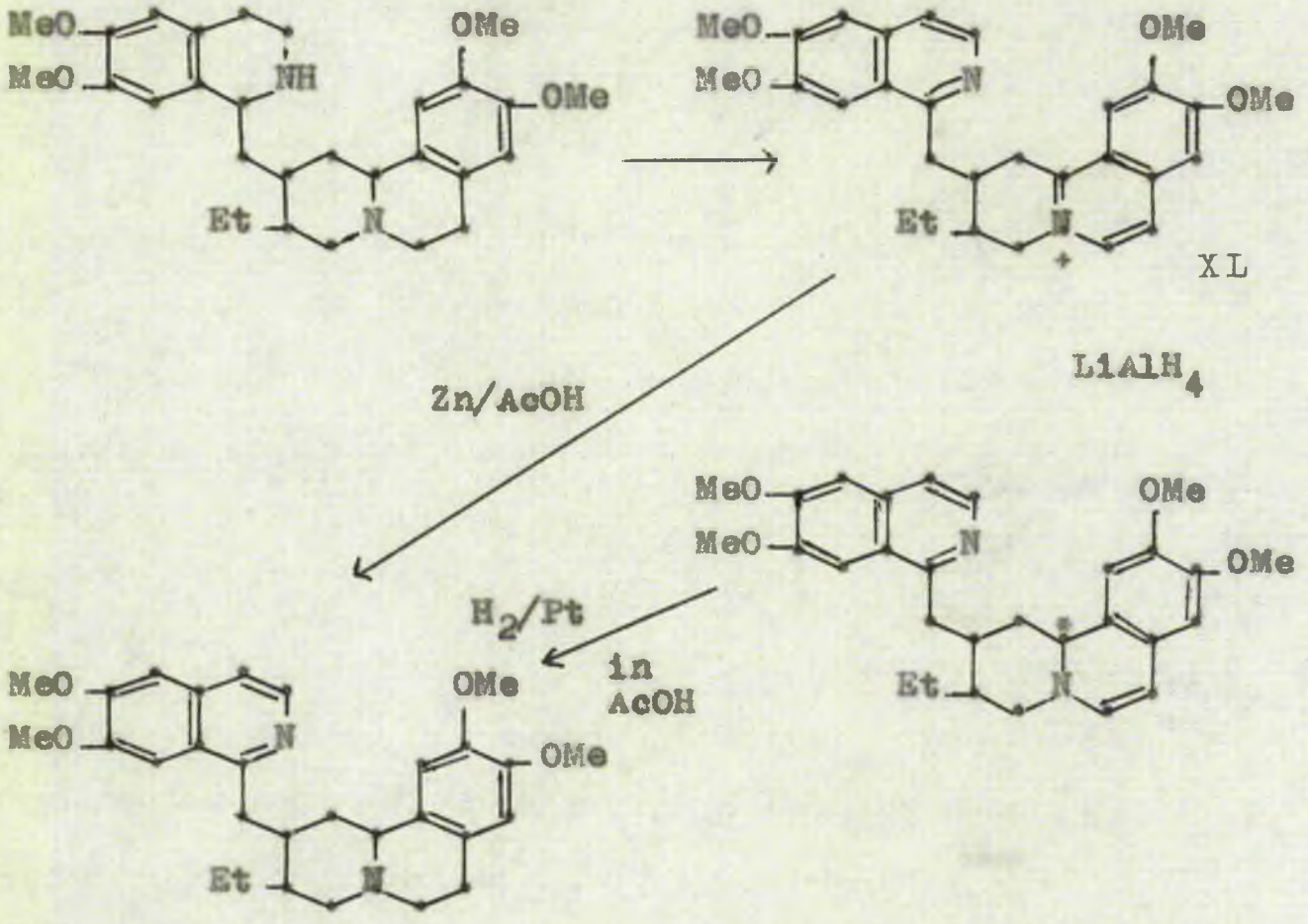
McEwen and Tietz in their reinvestigation of the reduction of rubremetine found that the second component isolated by Karrer and Rüttner was identical with α-dihydrorubremetine. Further they observed that if the catalytic reduction of rubremetine was continued for 100 hours after the initial uptake of one mole of hydrogen, a second mole was absorbed and the product obtained was a mixture of α-dihydrorubremetine and tetrahydrodehydroemetine After absorption of one mole of hydrogen they isolated α- and β-dihydrorubremetine.

Structure of Rubremetine

A structure had to be ascribed to rubremetine which would accord with the variety of reduction products. Pyman's initial formulation[13] in which the properties of rubremetine were attributed to formation of an amidine linkage was based on an incorrect formula for emetine but three other formulae have since been proposed.

Karrer[32] suggested that rings B and E of emetine were fully aromatised leading to structure (XL). The reduction of rubremetine was then explicable on the

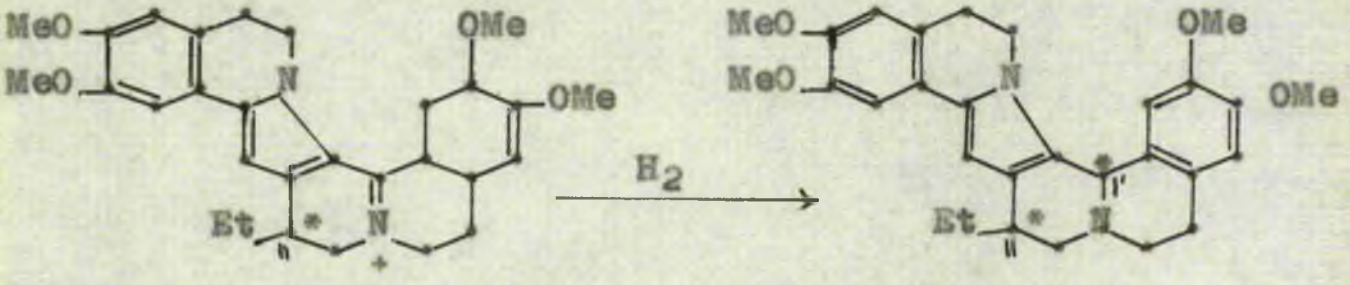
relative ease of reduction of an isoquinoline and isoquinolinium ring. Whereas the former is stable the latter is readily reduced. Secondly, lithium aluminium hydride had been shown[53] to reduce isoquinolinium rings only as far as a 1:2-dihydroisoquinoline. Hence, the following sequence of reactions was visualised in the reduction of rubremetine.



Tetrahydrodehydroemetine would thus seem to be a stereoisomer of emetamine.

However, emetamine on oxidation does not give rise to rubremetine but to a related compound which has been termed rubremetamine. Also the reduction with lithium aluminium hydride would introduce a new centre of asymmetry whence two reduction products would have been isolated, whereas, in fact, only one is obtained. Each of these factors cast doubt on the correctness of Karrer's structure.

Openshaw, Battersby and Wood[54] had suggested an alternative structure(XLI) for rubremetine. In this the colour of the compound can be attributed to a cyanine dye type of structure and the resistance to reduction beyond the dihydro derivative (XLII) to the stability of the pyrrole nucleus, evidence for which had already been obtained.



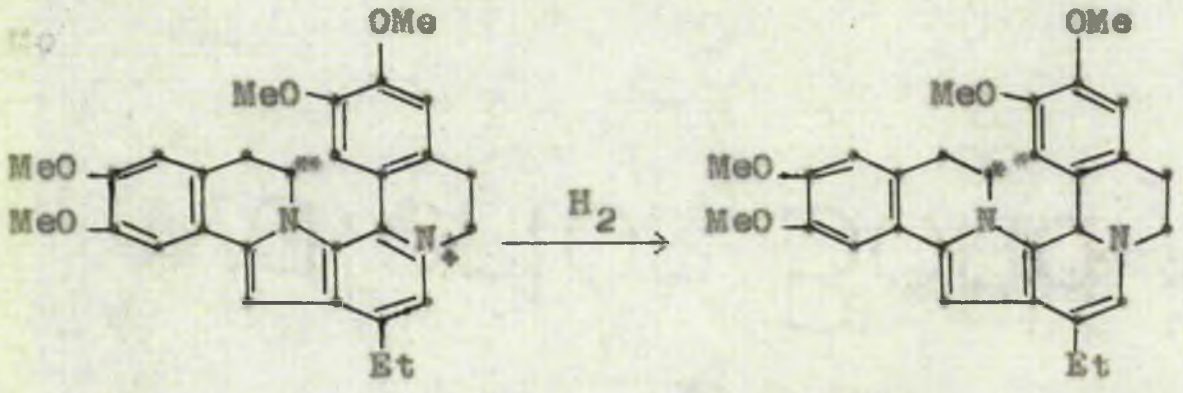
XLI XLII

Rubremetine still possesses a centre of asymmetry at C 11. Reduction introduces a new centre at C-1' whence α - and

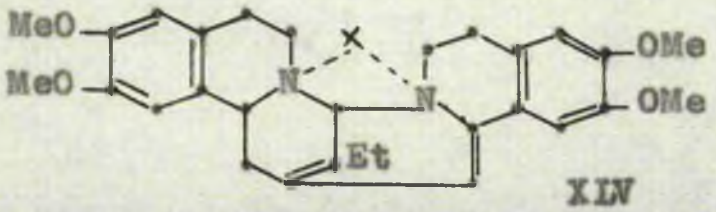
-dihydrorubtemetine are diastereoisomers. The nature of tetrahydrodehydroemetine remained unexplained but it was known to possess a similar ultraviolet absorption to the dihydro-compounds.

Karrer objected to structure (XLI) on the grounds that it possessed two dihydroaromatic rings which was an illogical end product to a dehydrogenation reaction. This objection was countered by citing the stability contributed to the system by resonance.

Woodward had also suggested[52] that rubremetine might have the structure XLIII in which all the centres of asymmetry have been destroyed and the optical activity of the molecule was ascribed to steric hindrance at the positions marked with an asterisk. The dihydro compounds would then have the structure XLIV



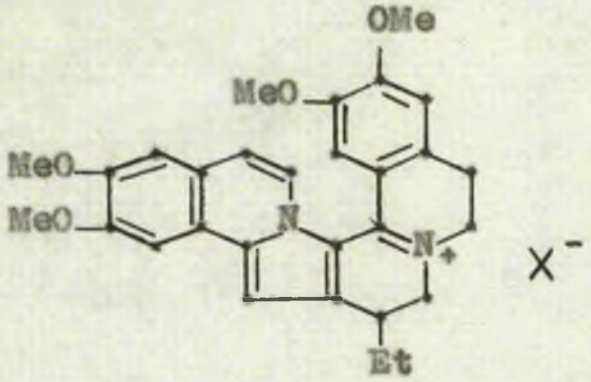
XLIII XLIV



XLV

Preobrazhenskii, when reporting the total synthesis of emetine[55], proposed that rubremetine might have the formula XLV, but this structure can be discounted as it is a meta bridged structure and does not possess a pyrrole ring.

Thus, when the work described in this thesis was begun, two formulae were being postulated for rubremetine, XLI and XLIII, and the nature of tetrahydrodehydroemetine was unknown. Rubremetamine was presumed to have the structure (XLVI) or the related Woodward structure.



Rubremetamine XLVI

THEORETICAL

I. A systematic examination of the total alkaloids of Ipecacuanha

In the earlier review of the biogenesis of isoquinoline alkaloids it has been pointed out that the formation of emetine from diphenylalanine afforded an example of a Woodward-type fission of a dihydroxy-aromatic ring. There existed, however, no evidence to support this mechanism and in the hope that alkaloids closely related to the postulated intermediates might be isolated, a detailed investigation of the alkaloidal material in the ipecacuanha plant has been undertaken.

The non-phenolic alkaloids emetine, o-methylpsychotrine and emetamine have hitherto been isolated[30, 31] from the crude mixture of bases as a consequence of the fact that they occur as the major constituents and form highly insoluble salts. Such a process of isolation would be unlikely to afford alkaloids which either form soluble salts or are present in minute quantity. A fractionation of the bases either by chromatography or counter-current distribution appeared more likely to

be successful in revealing new alkaloids. Although no comparable detailed fractionation had been undertaken at the time this investigation was being considered, counter-current distribution had been employed to separate the major bases occurring in several alkaloidal extracts[55].

At the same time it became necessary to isolate the alkaloid emetamine from natural sources as attempts to obtain it by dehydrogenation of the readily accessible emetine and o-methylpsychotrine in a parallel manner to that described by Ahl and Reichstein[36] had proved unsuccessful. Since Pyman had employed a modified form of counter-current distribution in his original separation of o-methylpsychotrine and emetamine[31] it was natural to favour that method in this study. Consequently, the alkaloidal content of Cephaelis Ipecacuanha was examined by a counter-current distribution technique.

Two sources of the alkaloid were available. The first was the bismuth iodide complex as which the residual alkaloids are precipitated after the crystallisation of emetine hydrobromide in the commercial process for isolating emetine. The second source was the mother liquors in the above process prior to the addition of bismuth iodide.

Each of these sources contained only the non-phenolic alkaloids extracted from the plant. A more detailed study was made of the bases stronger than o-methylpsychotrine isolated from the first source and of the bases weaker than o-methylpsychotrine from the second source.

Preliminary investigations indicated that at pH 6.4 the alkaloids were separated, when distributed between ethyl acetate and an aqueous phosphate buffer, into three fractions which corresponded to the three known non-phenolic alkaloids but there was evidence of a further alkaloid intermediate in basicity between emetamine and o-methylpsychotrine (Graph 1). This initial separation was effected by distributing the crude bases between one litre phases of ethyl acetate and half-molar aqueous buffer solutions but the more detailed fractionation was carried out successively in a 14-tube, 100 ml.phase and 100-tube, 10 ml.phase, Craig counter-current distribution apparatus.

In a detailed refractionation of the initial fractions (schemes 5 and 6) emetine (K=0.80 at pH 6.9), o-methylpsychotrine (K = 3.2 at pH 6.8) and emetamine (K = 1.94 at pH 5.7) were obtained in a pure state. The emetine

was identified as emetine hydrobromide and N-benzoyl emetine whilst the other two alkaloids were characterised as the free bases and their hydrogen oxalates.

No alkaloids intermediate in basicity between o-methylpsychotrine and emetine were identified.

Two new alkaloids were isolated which appear to be less basic than o-methylpsychotrine from consideration of the position in which they distribute. They have been termed protoemetine (K = 1.7 at pH 6.4) and Ipecac.alkaloid D_2 (K = 0.78 at pH 5.7).

Protoemetine

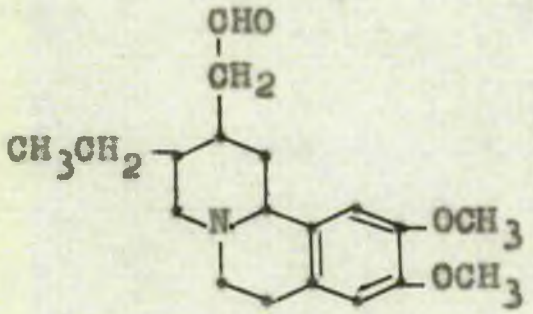
The alkaloid occurs to the extent of 0.002% of the dry root. It formed a crystalline perchlorate and was isolated and purified through this salt as the free base was found to be unstable.

Examination of the ultra violet absorption of the base indicated the presence of a simple veratryl residue. Mercuric acetate oxidation of the base gave a compound which possessed the characteristic absorption of a 3:4 dihydroisoquinoline whence it appeared that a 6:7-dimethoxy-tetrahydroisoquinoline system was present in the molecule. Since no N-benzoyl derivative was formed the nitrogen was tertiary in nature.

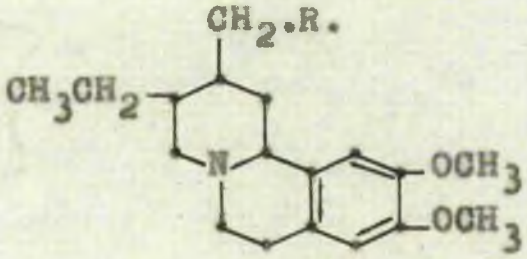
An equivalent weight determination showed the perchlorate to have a molecular weight of 498-506. Furthermore, hydrogen was absorbed upon catalytic hydrogenation and if the uptake of hydrogen was equivalent to 1 mole then an equivalent weight of 498 was indicated.

Since all the previous alkaloids which had been isolated from ipecacuanha had been inter-related the initial assumption was that this alkaloid would also be related. However, initially no agreement could be achieved between any postulated structure, the elemental analyses and the equivalent weight determinations and work on the identification of the alkaloid was temporarily suspended. The lack of agreement which is noted above was later explained when it was found that the perchlorate tenaciously retained water of crystallisation.

A subsequent examination of the infra red absorption spectrum indicated that an aldehyde group was present in the molecule. This was confirmed by Battersby and Harper[56] who proposed, on the basis of their analytical figures, the structure XLVIII for the alkaloid. Proof of this structure was forthcoming when the latter workers dehydrated the oxime of XLVIII to the nitrile (XLIX;R = CN)



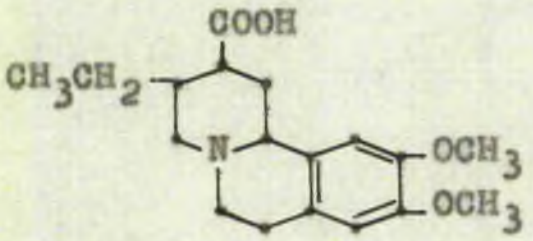
XLVIII



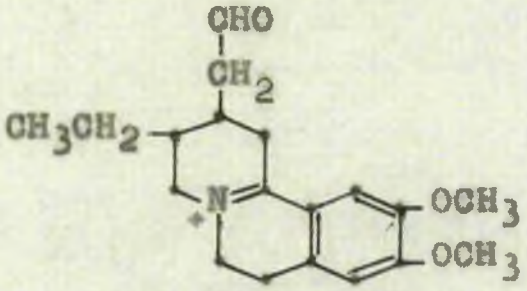
XLIX

which hydrolysed to give an amino acid (XLIX; R = COOH) identical with that which had previously been obtained by Arndt-Eistert homologation of L[57]. The degradation of o-methylpsychotrine to L is described later in this thesis.

This structure explains both the mercuric acetate oxidation product which must be (LI) and the observed uptake of one mole of hydrogen on catalytic hydrogenation when only the aldehyde group will be reduced.



L



LI

Ipecacuanha alkaloid D_2

This alkaloid occurs as only a very minor constituent of the bases occurring in ipecacuanha and the identification has had to be attempted on less than 200 milligrammes of base.

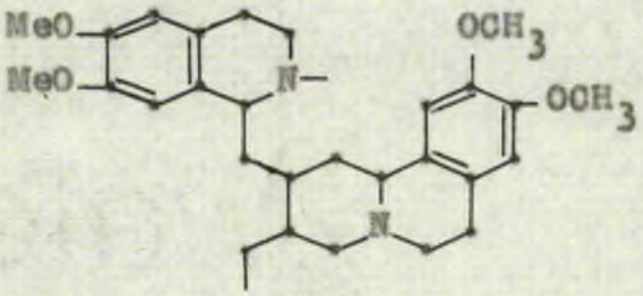
When the base was recovered from the hydrogen oxalate through which it was isolated, it crystallised from ethyl acetate and analysed for $C_{30}H_{40}O_5N_2$. A monopicrate and mono hydrogen oxalate were formed, analysis upon which confirmed the previous formula and showed that four of the oxygen atoms were present in methoxyl groups. An equivalent weight determination carried out on the picrate by the method of Spring[58] indicated an equivalent weight for the base lying between 530 and 560.

The ultra-violet absorption suggested that two veratryl groups were present. When mercuric acetate oxidation gave a compound exhibiting an ultra-violet absorption characteristic of the dihydroisoquinoline system together with an additional peak indicative of a residual veratryl absorption, the suggestion could be extended to cover a structure in which there was one tetrahydroisoquinoline group and one veratryl group.

The empirical formula suggested that the alkaloid was related to the other ipecacuanha bases. Emetine has the formula $C_{29}H_{40}O_4N_2$. Further evidence in support of this was forthcoming when the identification of propionic acid from Kuhn-Roth oxidation of the alkaloid[60] indicated that the ethyl group which occurs in emetine and the related alkaloids was also present in D_2.

The base did not absorb hydrogen on catalytic hydrogenation over platinum.

Initially it would appear to be safe to assume that the alkaloid has the basic emetine skeleton LII. The ultraviolet absorption indicates that there is no



LII

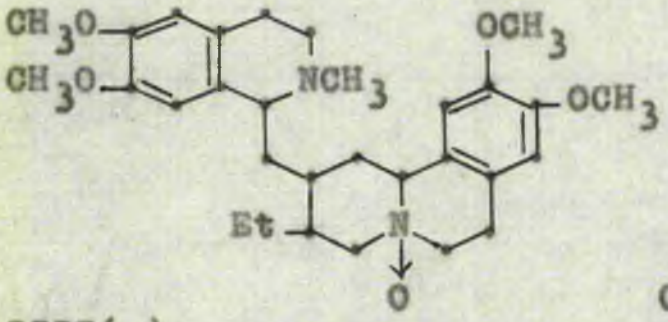
dihydroisoquinoline or isoquinoline ring in the molecule but suggests at least one tetrahydroisoquinoline ring. The empirical formula requires that one methylene group and one oxygen atom be introduced into the molecule. The methylene group can best be accommodated as an N-methyl

group and examination of the infra-red spectrum gave weight to the presence of two tertiary nitrogen atoms as there was no evidence of an N-H bond. At the same time it was apparent that the oxygen was not present as a carbonyl group, lactam or hydroxyl group.

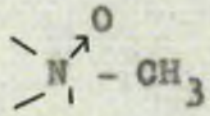
There existed a band at 1643 cm^{-1} which would point to the presence of a carbon-carbon double bond but the stability of the base to catalytic hydrogenation is at variance with the presence of such a linkage.

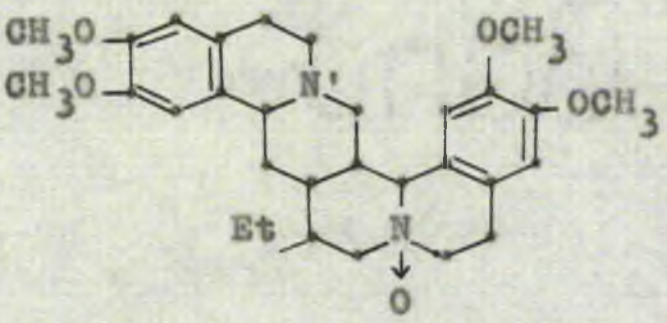
The oxygen would appear to exist either in an ether linkage or in an N-oxide. Aromatic heterocyclic N-oxides are stable to hydrogenation in ethanol and exhibit an infra-red absorption at 1255-1300 cm^{-1} and 847-872 cm^{-1} but there is no strong absorption at either of these frequencies and therewere no available figures for the infra-red absorption of an aliphatic N-oxide.

If the original premise that the alkaloid is related to emetine is correct, then it is difficult to conceive how the ether group can exist and the idea that the base is an N-oxide is preferred. Four possible formulae, LIII(a) and (b) and LIV (a) and (b), can be proposed.

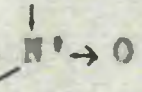


LIII(a)

(b) = 



LIV(a)

(b) = 

The existence of an N-oxide would not alone account for the fact that only a monosalt is formed but it is conceivable that in a base with the structure LIV the basic nature of the second nitrogen would be greatly reduced after the protonation of the first as in the case of a gem-diamine, e.g. eserine. Also, the existence of an N-oxide linkage would explain why only one of the tetrahydroisoquinoline groups is oxidised by mercuric acetate to a dihydroisoquinoline. Mercuric acetate oxidations require the co-ordination of mercury to a nitrogen atom[71] and if oxygen is already co-ordinated to the nitrogen of one ring no oxidation could take place

in that ring.

The instability of the free base to light would also be accounted for as the photolytic decomposition of N-oxides is well known[59].

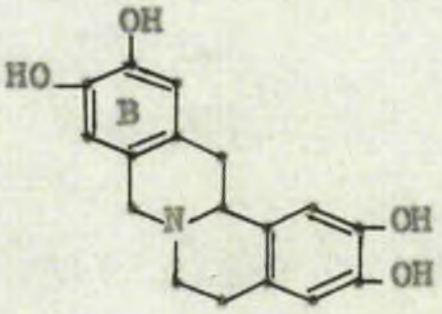
In all this work the recovery of bases from the countercurrent distributions has been less than expected. Such a behaviour would be explicable on the basis of N-oxides being present among the alkaloids as the N O groups would confer considerable water solubility on the bases and in the water washing of the organic extracts, which has been general practice, there could have been a considerable loss of alkaloidal material.

With the limited experimental evidence and amount of base available it is impossible to decide whether these structures are correct but at this stage of conjecture a preference for LIV (a or b) must be stated.

Further indications of the structure could be gained by an N-Me determination, catalytic reduction in acetic anhydride solution, comparison of the infra-red spectrum with that of the N-oxide of N-methyl-emetine and the measurement in alkaline solution of the ultra-violet absorption of the mercuric acetate oxidation product.

Significance of protoemetine and ipecacuanha alkaloid D_2

Protoemetine is the first instance in which an alkaloid possessing a free aldehyde group has been isolated from a natural product. However, the main significance of the alkaloid lies in the support which it affords for the biogenetic scheme for emetine which has been discussed in the section of biogenesis (p. 14) as condensation of protoemetine with one molecule of dihydroxyphenylalanine will lead directly to the emetine skeleton. At the same time, it would appear probable that one side chain in the product from the fission of ring B of the protoberberine XI, is fully reduced to an ethyl group before and not after the subsequent condensation.



XI

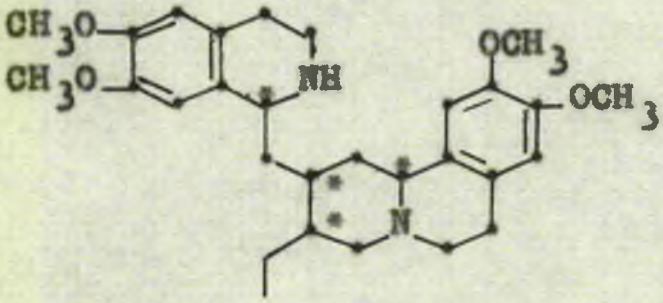
In this investigation it was hoped that nor-coralydin, the tetramethyl ether of XI, might be isolated but it was not obtained.

The possibility that D_2 may be an N-oxide suggests that the alkaloidal extract should be reduced in acid solution before any attempt is made to isolate the free bases. Several instances are known in which a significant increase in alkaloidal content has been observed after such a treatment and this has been shown to be due to the reduction of N-oxides. The existence of N-oxides in the plant is believed to point to the fact that the alkaloids act as an oxidation-reduction system and evidence for this is to be found in the fluctuating ratio of free alkaloid to N-oxide according to whether the plant is in a state of rest or growth. In the latter, there is always a greater N-oxide content. As the role of alkaloids in plant metabolism is as yet undecided, this would appear to be an interesting field for further investigation.

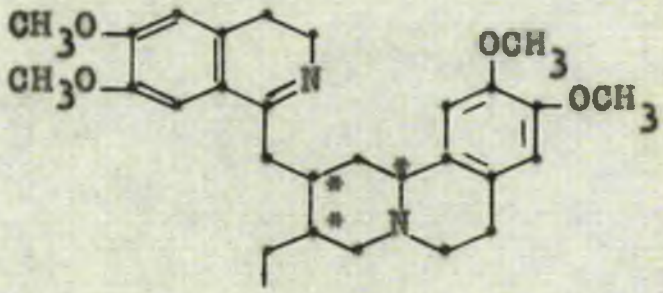
II. An approach to the stereochemistry of emetine.

Configuration at C_{10}.

In the emetine molecule (LV) there are four centres of asymmetry situated at C_1, C_{10}, C_{11} and C_1'. The configuration at C_1 should be obtainable by degradation

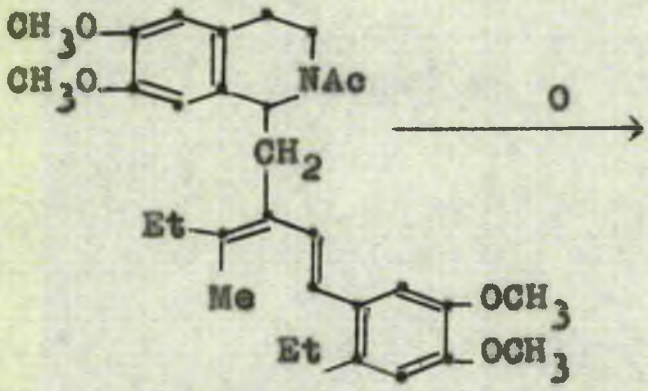


LII

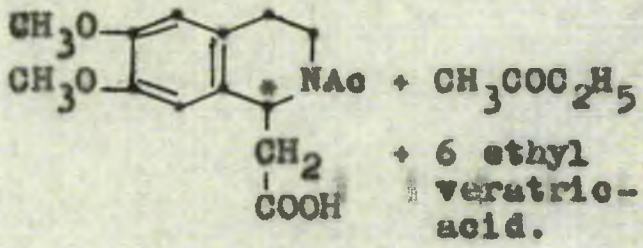


LV

of the molecule to LVII by oxidation of the end product LVI from successive Hofmann degradations of N-acetylemetine where the double bond introduced in the initial step has been reduced.



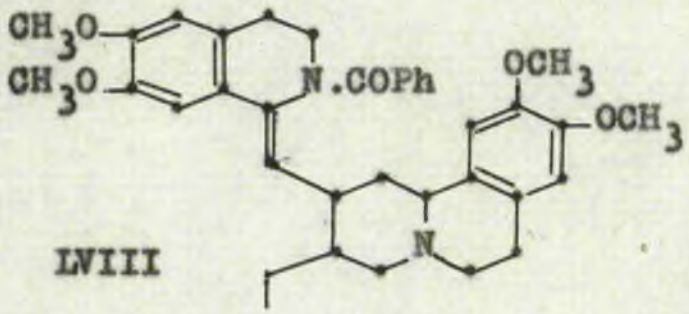
LVI



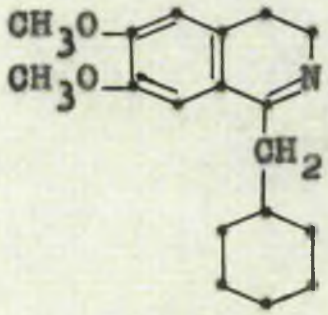
LVII

LVII retains the asymmetry of C_1 in the emetine molecule and it should be possible to relate this compound to one of the tetrahydroisoquinoline alkaloids whose stereochemistry at C_{11} is known.

In order to examine the stereochemistry at the other centres it was thought desirable to cleave the molecule between C_9 and C_1 whereby the asymmetric centres would be retained in a reduced benzoquinolizidine system in which they would be accessible to individual study. However, as has already been indicated, no degradation of emetine or its associated alkaloids has been carried out from which the tricyclic system has been isolated.



LVIII



LIX

Now although o-methylpsychotrine has lost the asymmetric centre at C_1 it retains the other three centres in the same configuration as in emetine as is evidenced by the reduction of o-methylpsychotrine to emetine, and in N-benzoyl-o-methylpsychotrine, LVIII, the double bond is in the desired exocyclic position for a cleavage of the molecule. Karrer[32] has isolated N-benzoyl-corydaldine from the oxidation of LVIII with perphthalic acid or ozone but in poor yield. If the yield of N-benzoyl-corydaldine could be improved this oxidation should also afford the desired benzoquinolisidine. With this in view the oxidative cleavage of 1-cyclohexylmethyl-3:4-dihydro-6:7-dimethoxyisoquinoline, LIX, was studied.

LIX was prepared by a Bischler-Napieralski ring closure of the amide resulting from the condensation of homoveratrylamine with 1-cyclohexylacetic acid chloride. The parent acid of the latter compound was obtained by the acid hydrolysis of cyclohexylacetonitrile since apparatus was not available at the time for the more obvious catalytic reduction of phenylacetic acid. The nitrile in turn had been prepared by the specific catalytic hydrogenation over a palladium on strontium carbonate

catalyst of cyclohexene acetonitrile which resulted from the condensation of cyclohexanone and cyanoacetic acid in the presence of piperidine. The base formed a crystalline perchlorate but did not form a hydrogen oxalate.

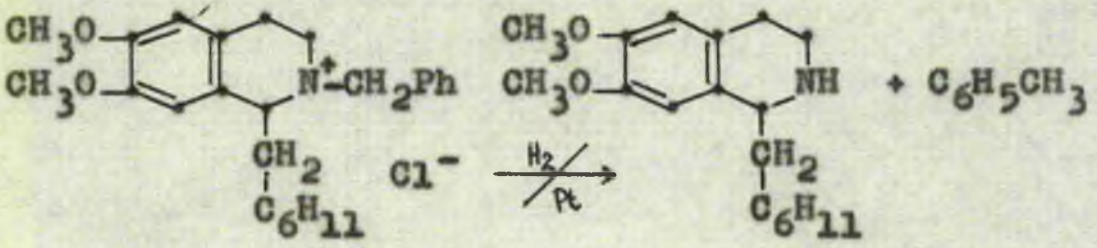
Both LIX and its N-benzoyl derivative were stable, even on gentle warming, to oxidation with potassium permanganate in aqueous acetone solution, whereas, N-benzoyl-emetine was rapidly attacked under similar conditions.

In view of the apparent stability of the N-benzoyl 3:4 dihydroisoquinoline the oxidative cleavage of this type of compound was not pursued. Instead it was decided to form a quaternary salt of LIX which in the presence of an excess of alkali would yield an iso-base susceptible to mild oxidation[61].

In considering the extension of this reaction to o-methylpsychotrine it was apparent that it would be preferable to form a quaternary benzyl salt since reduction of the oxidation products would regenerate the tertiary nitrogen in the quinolizidine system due to hydrogenolysis of the benzyl group. Consequently

the benzyl chloride of LIX was prepared. The resultant quaternary chloride LX was preferably purified as the iodide which crystallised from ethanol.

An attempt to remove the benzyl group from the quaternary iodide by catalytic hydrogenolysis was unsuccessful. However, when the iodide was reconverted to the chloride by treatment with silver chloride it was quantitatively reduced to 1-cyclohexylmethyl-6:7-dimethoxy-tetrahydroisoquinoline LXI.

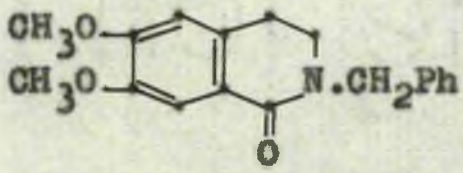


LX LXI

It materialises that iodide ions generally stabilise quaternary benzyl compounds to hydrogenolysis as several instances are recorded of the stability of benzyl iodides whereas the corresponding chlorides and hydroxides were readily reduced.(62).

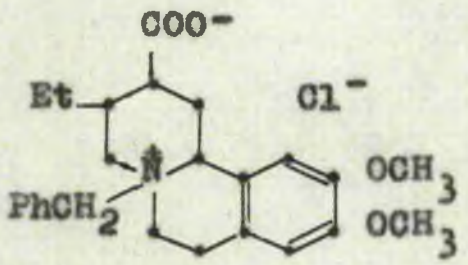
The oxidation of the iso-base was carried out at 0-5° in the presence of an excess of alkali. When the

theoretical amount of potassium permanganate was added to a solution of LX in aqueous acetone the isocarbostyril (LXII) was isolated in 37% yield but only a very small amount of acidic material was obtained. It was shown that aqueous alkaline acetone was rapidly oxidised by permanganate and aqueous dioxan was substituted as the solvent after which the isocarbostyril and cyclohexyl-acetic acid were obtained from the oxidation in yields of 49% and 48% respectively.

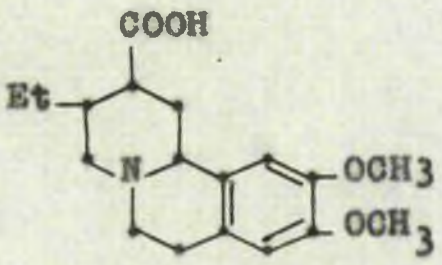


LXII

o-Methylpsychotrine formed a dibenzochloride which was oxidised in alkaline dioxan solution with the theoretical quantity of aqueous permanganate. From this oxidation the isocarbostyril LXII was isolated in 64% yield. It had been hoped that the other product of the oxidation would be the betaine LXIII which upon hydrogenation in acid solution would be converted to the amino acid LXIV through hydrogenolysis of the benzyl group.

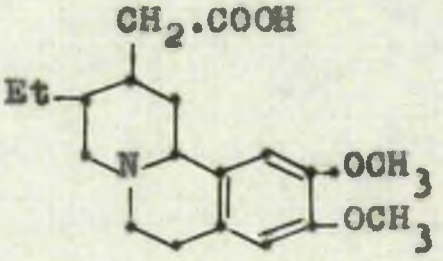


LXIII



LXIV

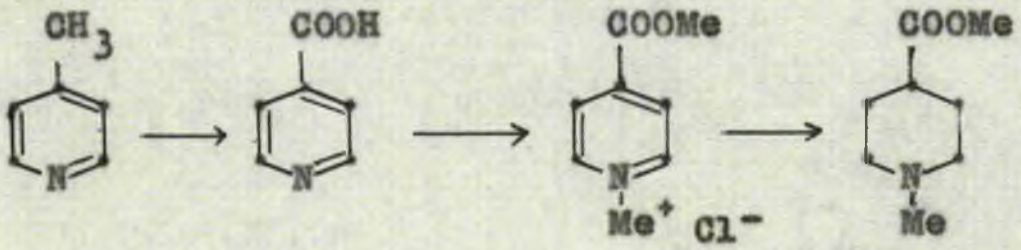
As anticipated, a beautifully crystalline amino acid was obtained which analysed for $C_{18}H_{25}O_4N.1H_2O$. The ethyl ester of this amino acid was optically active and it was shown that the carboethoxy group was in the equatorial configuration when the amino ester was stable to epimerisation with sodium methoxide. However, the stable configuration might have been attained in the degradation. In order to decide this point, it became necessary to synthesise o-methylpsychotrine from the amino acid, LXIV, which in turn resolved itself into the problem of synthesising the homologous amino acid, LXV, from LXIV with retention of the configuration. The subsequent condensation of the acid, as the acid chloride,



LXV

or ethyl ester, with homoveratrylamine followed by Bischler-Napieralski ring closure of the amide are standard reactions. If o-methylpsychotrine were obtained in the synthesis then the isoquinolyl group at C_{10} in the emetine molecule must be equatorial.

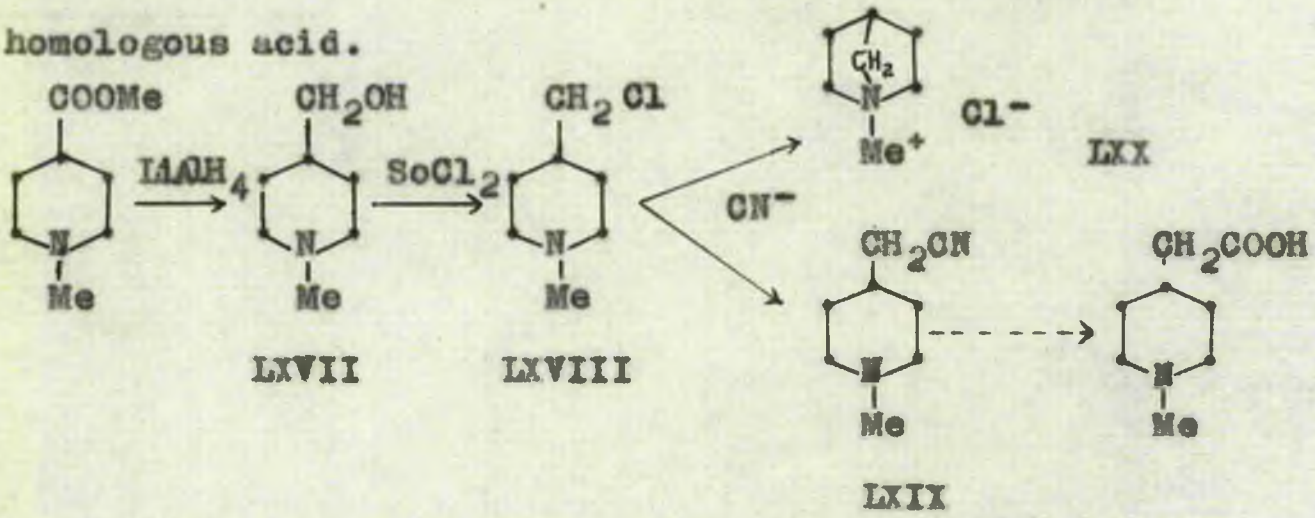
For the initial experiments leading to the homologation of LXIV, 1-methyl-4-carbomethoxy-piperidine, LXVI, was used as a model compound. It was prepared from -picoline which was oxidised to iso-nicotinic acid in 53% yield by potassium permanganate. The desired product was obtained by catalytic reduction of the methochloride of methyl nicotinate.



LXVI

The sequence of reactions envisaged in the homologation involved reduction of the ester group with lithium aluminium hydride to the amino alcohol, LXVII, which would be readily converted to the amino chloride, LXVIII, on

treatment with thionyl chloride. Cyanide ion would be expected to react with the chloride to form the nitrile, LXIX, which, upon hydrolysis, would give the desired homologous acid.



Although the chloride, LXVIII, was obtained in excellent yield when the subsequent conversion of the halide to the nitrile was attempted only a minute quantity of base was recovered. Obviously the quaternary salt, LXX, had been formed.

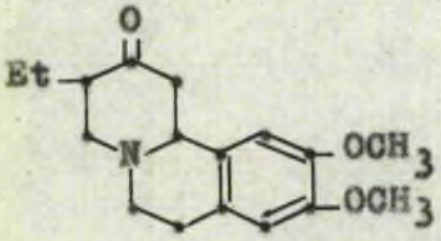
In a similar reaction with 3-hydroxymethylpiperidine, Merchant and Marvel[63] first formed the N-benzoyl derivative after which a 40% conversion to the nitrile was obtained on boiling with sodium cyanide in ethanol for 48 hours. As it is impossible to form a benzoyl derivative of LXVIII it was decided to prepare the quaternary benzyl

chloride in the hope that the conversion of the halide to the nitrile would proceed smoothly as the nitrogen would already be quaternary. On this occasion when the benzyl group was removed by hydrogenolysis, LXVIII was recovered unchanged. When subsequent attempts to carry out the reaction using the quaternary iodide and mercuric cyanide were also unsuccessful, this method of homologation was rejected.

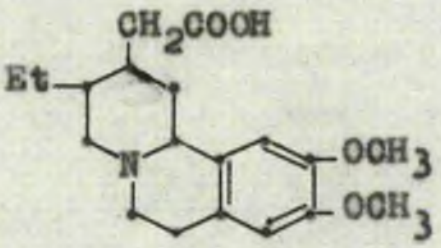
Configuration at C_{11}

Whilst attempting to discover the conformation at C_{10} in the emetine molecule, the degradation of the molecule to study the other two asymmetric centres was also investigated.

Barbier-Wieland degradation of the ethyl ester of the amino acid, LXIV, should result in the formation of the amino ketone, LXXI, through which it should be possible to equilibrate the adjacent ethyl group at C_{11}.



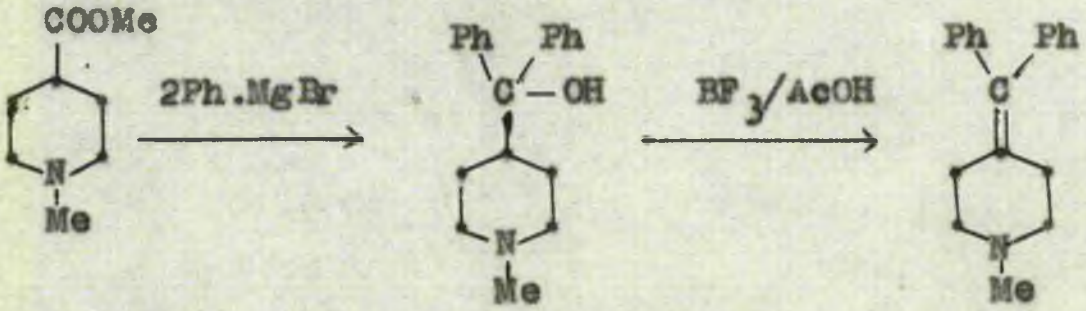
LXXI



LXXII

If subsequent conversion of the ketone with the ethyl group in the stable configuration to the amino acid, LXV, results in the formation of the same acid as that obtained by homologation of the amino acid, LXIV, then the configuration of the ethyl group in the original molecule would be unequivocally established.

The sequence of reactions was first studied with 1-methyl-4-carbomethoxypiperidine, LXVI. From the reaction of phenylmagnesium bromide with LXVI the carbinol, LXXIII, was obtained in quantitative yield. This tertiary alcohol was resistant to dehydration by the action of



LXXIII LXXIV

acetic anhydride in acetic acid but was readily converted to 4-benzhydrylidene-N-methyl-piperidine, LXXIV, by warming with boron trifluoride in acetic acid[64]. At the same time the intermediate boron trifluoride complex was isolated. With a melting point of 208-209° this

complex possesses a higher melting point than any previously reported for a boron trifluoride co-ordination complex with an organic compound[65].

The benzhydrylidene, LXXIV, proved remarkably stable to oxidation with permanganate and ozone. When it also failed to absorb hydrogen in a catalytic reduction it was doubted whether the benzhydrylidene structure was correct. However, that it had been produced in the dehydration was evidenced by a comparison of the ultra-violet absorptions of the carbinol and the product of dehydration. The ultra-violet absorption of benzhydrylidene compounds has been reported by Braude and Coles[66] and a similar absorption was observed in the case of LXXIV. The elemental analysis also confirmed the benzhydrylidene structure.

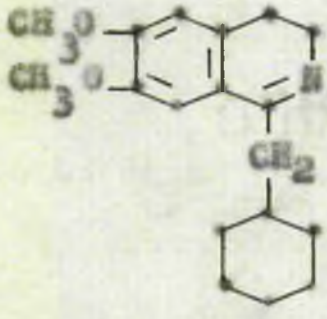
As it has proved impossible oxidatively to cleave the double bond to yield the 1-Me-4-piperidone in the case of the model compound the method was not attempted with the ethyl ester of LXIV.

The stability of the benzhydrylidene to oxidation by peracetic acid was reported by Lyle and Lyle[77] whilst the foregoing work was being carried out. These authors had prepared the compound by the same route but used modified conditions and dehydrating agents.

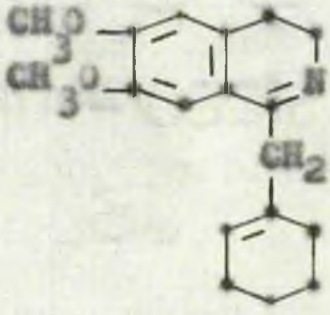
Speculation as to the nature of a by-product isolated in the preparation of 1-cyclohexylmethyl-3:4-dihydro-6:7-dimethoxy-isoquinoline, LXIII.

In the initial preparation of 1-cyclohexylmethyl-6:7-dimethoxy-3:4-dihydro-isoquinoline a compound was isolated in 14% yield as a crystalline hydrogen oxalate. It was later shown to be different from the desired product which can be characterised as the perchlorate but which does not form a crystalline hydrogen oxalate.

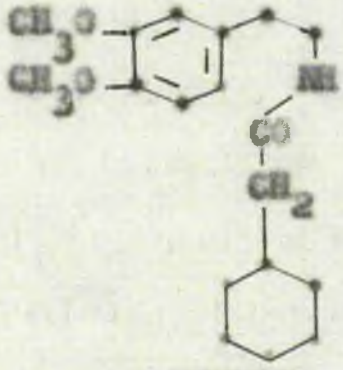
This by-product possessed an identical ultra-violet absorption to LXIII and also absorbed one mole of hydrogen



LXXV



LXXVI



LXXVII

on catalytic reduction. However, analysis indicated

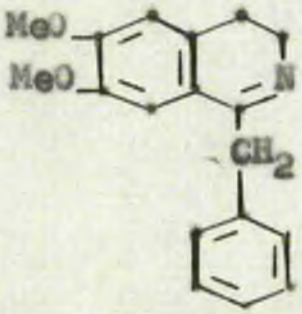
that it contained four hydrogen atoms less than LXIII which precluded the possibility that the impurity was the 7:8-dimethoxy-3:4-dihydro-isoquinoline formed by cyclisation to the ortho-position.

On this occasion, the stages of hydrolysis and reduction in the preparation of the cyclohexyl acetic acid had been reversed and doubt existed as to whether a quantitative uptake of hydrogen had occurred in the reduction of the cyclohexene acetic acid. Consequently, the latter acid may have been present as an impurity in the cyclohexyl acetic acid which was used to prepare the amide, LXVII, to be used for the Bischler-Napieralski ring-closure to LXV. If this were so then the expected impurity would have been 1-($\Delta^{1'}$-cyclohexenyl)-methyl-3:4-dihydro-6:7-dimethoxy-isoquinoline, LXXVI. However, this compound would have absorbed two moles of hydrogen on catalytic hydrogenation.

It is impossible to conceive any reaction of LXXVI which would result in the formation of a compound with the desired properties and possessing four hydrogen atoms less than LXXV but several possibilities exist where two or six hydrogen atoms have been lost.

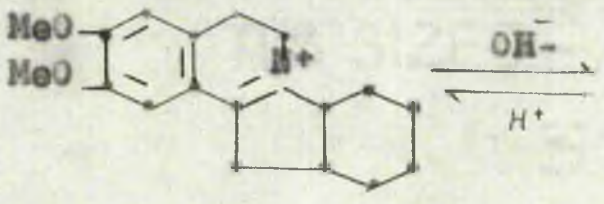
The latter situation could arise by complete aromatisation of the cyclohexane ring but the compound

was not identical with 1-benzyl-3:4-dihydro-6:7-dimethoxy-isoquinoline, LXXVIII.

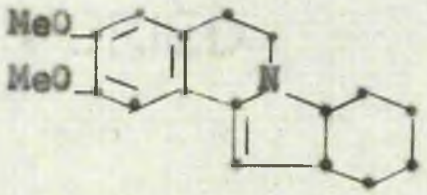


LXXVIII

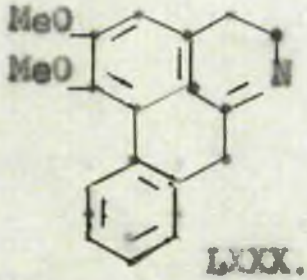
Alternatively, LXXVI could react further to give rise to LXXIX, LXXX or LXXXI. The quaternary dihydro-isoquinoline, LXXIX(a), will form an iso-base, LXXIX(b), in alkaline solution in contrast with the other two structures. As measurement of the ultra-violet absorption in acid and alkaline solution[68] indicated that an iso-base was not formed, structure LXXIX can be rejected.



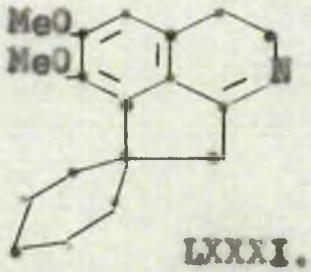
LXXIX(a)



LXXIX(b)



LXXX.

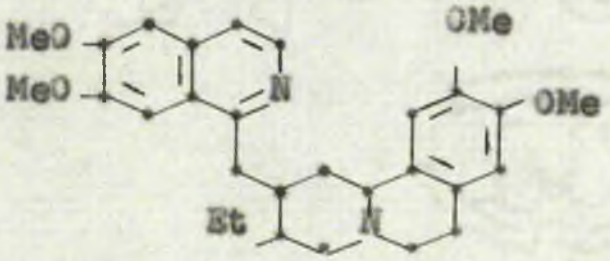


LXXXI.

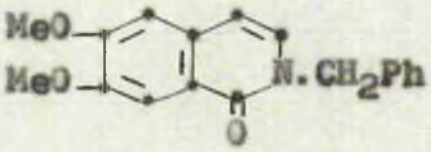
In considering formulae LXXX and LXXXI, the reaction of the double bond in LXXVI with the 8-position in the dihydro-isoquinoline ring is parallel to the alkylation of benzene through reaction with an alkene. It has been shown by Ipatiev[69] that the latter reaction is catalysed by 80-85% phosphoric acid and it is reasonable to suppose that the phosphorus oxychloride employed in the ring closure would act similarly. Both the 5- and 8- positions in a 6:7-dimethoxy-3:4-dihydroisoquinoline system are equally activated towards electrophilic substitution but on steric grounds only the 8-position would be available to react with the cyclohexenyl double bond. Again, by analogy with the alkylation of benzene the expected product would be the more highly branched isomer, LXXXII. Consequently, it is proposed that the by-product from the preparation of 1-cyclohexylmethyl-3:4-dihydro-6:7-dimethoxy-isoquinoline is LXXXI, a spiro compound involving a five-membered ring; but this structure cannot be accepted as proven until further investigations have been carried out using Δ^1- cyclohexenylacetic acid.

Emetamine

Emetamine has been assigned structure LXXXII, in which ring B of the emetine molecule is fully aromatic. Proof of this structure should be provided by applying the technique used previously to cleave the o-methylpsychotrine molecule to the emetamine molecule when it should be possible to isolate the isoquinolone, LXXXIII, if the postulated structure is correct.



LXXXII



LXXXIII

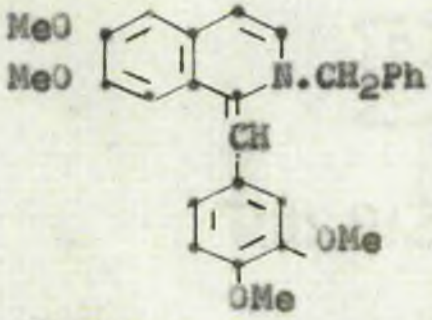
However, although emetine and o-methylpsychotrine are readily isolated from the plant in quantity emetamine is not. Ahl and Reichstein[36] had prepared emetamine by the catalytic dehydrogenation of emetine over a palladium charcoal catalyst at 180-190° and it was decided to prepare the minor alkaloid in a like manner.

In a series of catalytic dehydrogenations in which the base was intimately mixed with the catalyst and in others where boiling p-cymene and boiling caryophyllene were employed as solvents neither emetine nor o-methylpsychotrine yielded emetamine. In confirmation of an earlier observation by Wood[67], o-methylpsychotrine was obtained from emetine as the major product whilst from both a mixture of bases was isolated which possessed the same partition coefficient as emetamine.

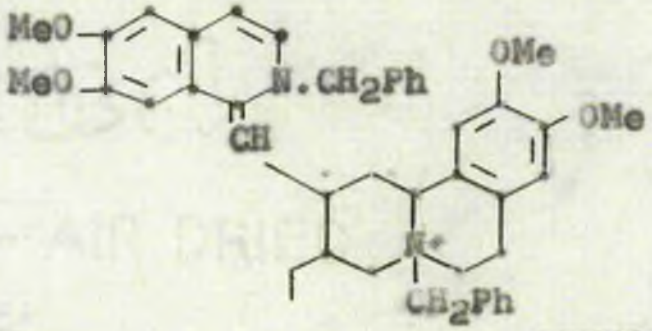
One of these, "A", melted at the same temperature as emetamine, both as the free base which crystallised from ether, and as the hydrogen oxalate. But, whereas emetamine is dextro-rotatory as the free base and laevo-rotatory as the hydrogen oxalate, "A" was dextro-rotatory in both states. "A" had a similar ultra-violet absorption to emetamine and was resistant to catalytic hydrogenation. On oxidation with mercuric acetate, "A" formed a red quaternary compound which an X-ray powder photograph proved to be identical with rubremetamine, the product from mercuric acetate oxidation of emetamine. To fit this evidence, "A" must be a stereoisomer of emetamine.

The emetamine which was necessary before the structural proof could be undertaken was eventually obtained in the systematic examination of the alkaloids present in the plant (p.51).

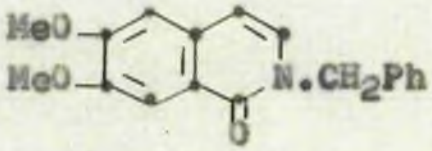
When the iso-base, LXXXIV, from the dibenzochloride of emetamine was oxidised in aqueous alkaline dioxan with the theoretical quantity of permanganate, 1-keto-2-benzyl-6:7-dimethoxy-1:2-dihydroisoquinoline, LXXXVI, was isolated. This compound was identical with that obtained from a similar degradation of papaverine benzochloride, LXXXV. Hence emetamine must contain an isoquinoline system in rings A and B. This confirms the structure of emetamine as LXXXII.



LXXXV.



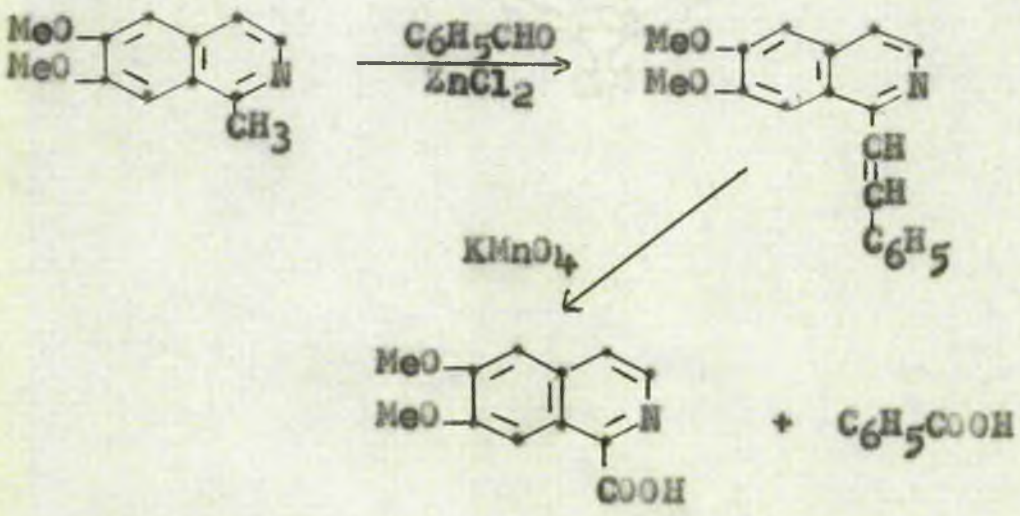
LXXXIV.



LXXXVI.

since the isolation of isoemetine from the reduction of emetamine with sodium in ethanol[13] had previously indicated that the alkaloid possessed the same stereochemistry as emetine in the rest of the molecule.

It had earlier been intended to vigorously degrade the emetamine molecule by oxidation, and, in connection with this, 6:7-dimethoxy-isoquinaldic acid was prepared by a novel route. 1-Styryl-6:7-dimethoxy-isoquinoline was prepared by the condensation of 1-methyl-6:7-dimethoxy-isoquinoline with benzaldehyde in the presence of zinc chloride as described by Campbell, Tipson and Elderfield[75]. The oxidation of the styryl compound with permanganate proceeds in high yield and the acid crystallises on adjustment of the solution to pH 4-5.



Rubremetamine

Emetine is oxidised by mild oxidising agents such as ferric chloride, bromine, iodine or mercuric acetate to rubremetine, a red crystalline quaternary salt, for which the structure LXXXVII is now accepted. Emetamine is also attacked by the same oxidising agents with the formation of a red quaternary salt, which by analogy with the oxidation product of emetine will be referred to as rubremetamine. Pyman (13) and Karrer (32) have shown that rubremetamine is not identical with rubremetine by comparing the melting points and ultra-violet and visible absorption spectra of the two compounds. However, on neither occasion was rubremetamine isolated and fully characterised. Rubremetamine has now been obtained crystalline and fully characterised.

Catalytic hydrogenation of rubremetine in ethanol buffered with sodium acetate yields a mixture of and dihydrorubremetine. Under similar conditions rubremetamine absorbed rapidly 0.97 mol. of hydrogen after which absorption ceased. The reduction products, which must be dihydro-derivatives, crystallised from ethanol as needles and buttons which were separated manually and purified by recrystallisation. The two compounds possess distinct melting points but identical

ultra-violet absorption and have been designated α- and β- dihydrorubremetamine. They are both reoxidised by mercuric acetate.

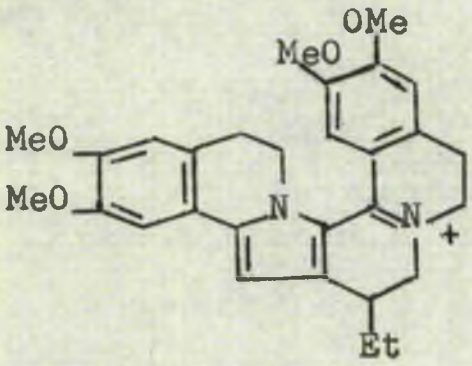
By comparison with rubremetine rubremetamine has been assigned the structure LXXXVIII. α- and β-dihydrorubremetamine would then have the structures LXXXIX (a) and (b), differing only in the spatial configuration of the hydrogen at C_1. A molecule of this type would be expected to be resistant to further reduction and to be readily oxidised.

An examination of the molecule of the dihydrorubremetamines indicates the presence of two distinct chromophores so that the ultra-violet absorption spectra of these compounds should consist of a veratryl absorption super-imposed upon a dimethoxybenzopyrrocoline absorption. The similarity (fig.11) between the actual absorption spectra and that of 3-methyl-7:8 benzopyrrocoline, XC, which has been prepared by Boekelheide (70) suggests that the benzopyrrocoline system is present in the dihydrorubremetamines and provides additional evidence for the postulated structures of rubremetine and rubremetamine.

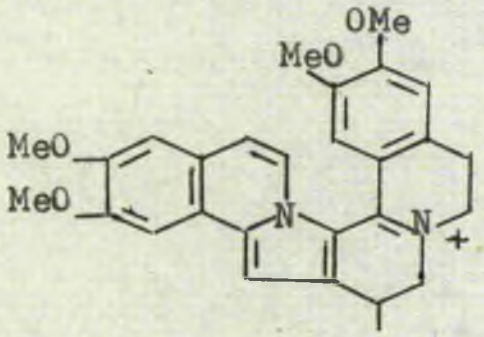
The oxidation of emetamine with mercuric acetate yielded on occasion small quantities of a yellow quaternary salt in addition to rubremetamine. Subsequently it was shown that this compound was a further oxidation product of rubremetamine. A similar type of compound was obtained from rubremetine but in much lower yield whence it would appear that rubremetine is more resistant to further oxidation than rubremetamine.

The absorption spectra of the oxidation products when measured over the range 2000-5000^{A} were more complex than the parent salts indicating the formation of a new chromophoric system. Unfortunately, as the work had to be terminated before the exact nature of the products could be determined only a qualitative comparison can be made between the curves in figs.9 & 10. However, an examination of the oxidation of emetine allows the postulation of structures for the two compounds.

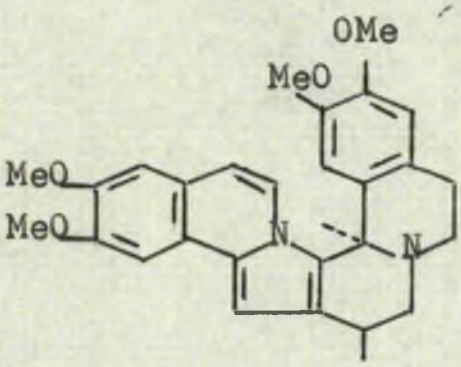
Leonard and co-workers (72) have studied the mercuric acetate oxidation of N-heterocyclic compounds and suggest the following mechanism for the reaction in the case of the introduction of α-β unsaturation into quinolizidine.



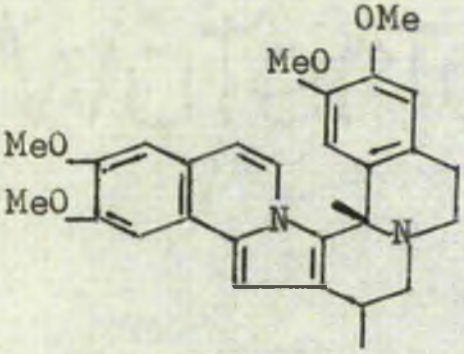
LXXXVII



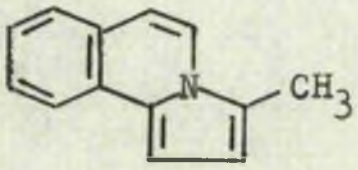
LXXXVIII



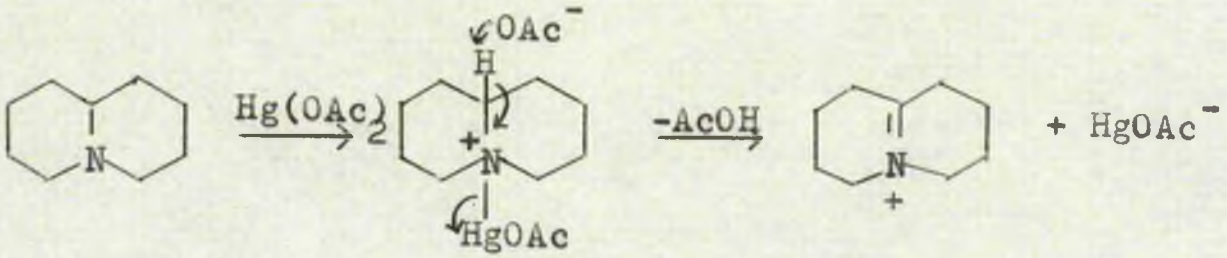
LXXXIX(a)



LXXXIX(b)



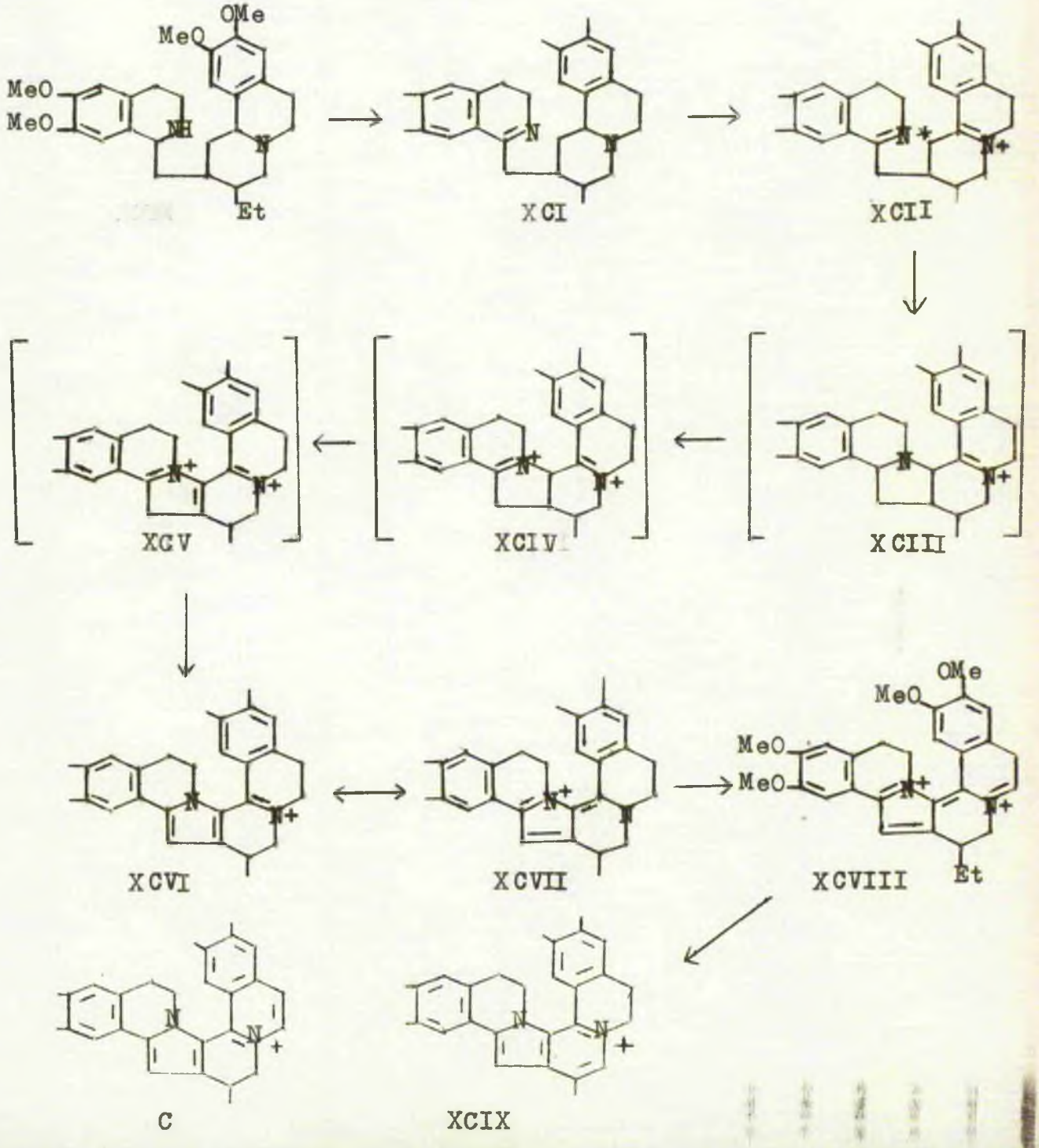
XC



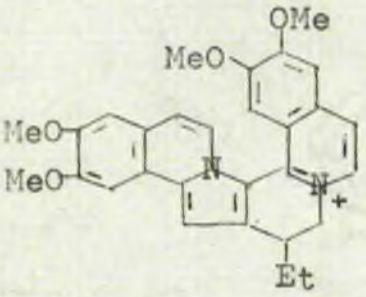
A similar reaction is postulated for the oxidation of tetrahydroisoquinolines with the initial reaction being the attack of the nitrogen bythe mercurous salt. In considering the formulae of the ultimate oxidation products of emetine and emetamine it is probable that the nitrogen atoms will be the initial sites attacked by the mercurous acetate and the reaction sequence outlined in scheme 7 is proposed for the oxidation of emetine by mercuric acetate.

The initial oxidation products, O-methylpsychotrine, XCI, and tetradehydroemetine, XCII, have been isolated. In the stage following the formation of tetradehydroemetine the nitrogen, N(a), would be expected to condense with the highly activated C_9 position (*) to give XCIII in what seems to be the rate-determining reaction since no compound has been isolated which has been shown to have the structure of any of the postulated intermediates. These, however, will only have a transient existence in the presence of excess mercuric acetate as XCIII will be readily oxidised to XCIV in which C_9 is now more highly activated than in

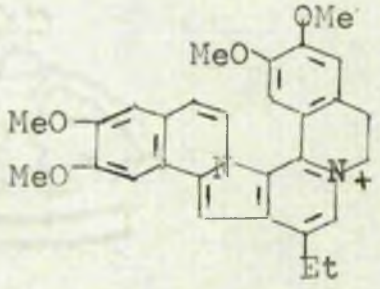
Reaction sequence in Mercuric Acetate oxidation of Emetine.



tetradehydroemetine so that C-mercuration could occur. Elimination of the mercuriacetate ion would result in XCV which by loss of a proton could rearrange to give rubremetine whose structure is a resonance hybrid of XCVI and XCVII. Further attack by mercuric acetate at the less hindered nitrogen could result in formation of XCVIII which again, on loss of a proton could rearrange to either XCIX or C. By analogy rubremetamine would be oxidised to CI or CII.



CI



CII

An explanation of the observation that rubremetamine is more readily oxidised than rubremetine may be found by examining the stereochemistry of the two compounds. For the elimination reaction to proceed the molecule must assume a planar configuration but Woodward[52] has suggested that steric interference between the hydrogen atoms at C_3 and C_8, prevents the rubremetine molecule readily assuming a planar configuration. Furthermore, the complete aromatisation of rings D or E in the suggested structure for the oxidation product should confer added rigidity to the molecule which will be under greater strain than that

of rubremetine. Rubremetamine, on the other hand, having lost one of the hydrogen atoms at C_3, possesses an almost planar configuration which will react more readily with mercuric acetate.

The deep red colour of the parent compounds has been attributed to the cyanine dye type structure in which the positive charge is shared between the two nitrogen atoms, but in the structures of the final oxidation products the charge would lie predominantly on $N_{(b)}$ since the contributing canonical structures wherein the charge lies on $N_{(a)}$ are either quinimoid or not fully conjugated. Although the whole molecule remains stabilised by resonance the wavelength of maximum absorption would be expected to move to a shorter wavelength. Thus the change from a deep red colour to a golden yellow would be adequately explained.

Experimental

The small-scale countercurrent distributions (up to 3g. of base) were carried out in a fully automatic glass machine possessing 100 tubes whose upper and lower phase volumes were each 10ml.. The distributions between 100ml. phases were carried out in a 16-tube machine constructed by the glass-blowing department at St.Andrews University.

All distributions were undertaken at 70° ± 3° F.. The tubes were numbered in the customary manner 0,1,2,.... and the upper phases transferred. Analyses were carried out by weight following evaporation of aliquots of the upper phase.

Solutions of organic solvents were dried over sodium sulphate and evaporations carried out under reduced pressure at temperatures of 40°C. or less.

Analytical samples were dried at 100°C. over phosphorus pentoxide in vacuo unless otherwise stated.

Preliminary examination of the alkaloids from the bismuth iodide complex.

The residual non-phenolic alkaloids, after the commercial extraction of emetine from the Ipecacuanha root, are precipitated as the bismuth iodide complex. The alkaloids contained in this were investigated

The bismuth iodide complex (6.05g.) was suspended in 2N sodium hydroxide solution (100ml.) and extracted with ether (3x300ml. followed by 3x100ml.). The combined extracts were washed with 2N sodium hydroxide solution and water. Evaporation of the dried solvent yielded a clear gum (1.26g) which was scattered equally in five tubes of the machine and distributed between ethyl acetate and a phosphate buffer of pH6.4.

The results of the weight analyses after 95 transfers, recorded in table 1 , are plotted in graph 1.

Table 1.

Tube No.	Wt. mg./ml.	Tube No.	Wt. mg./ml.	Tube No.	Wt. mg./ml.
3	2.52	43	0.63	85	0.14
6	2.70	48	0.09	88	0.32
8	2.62	53	0.02	89	0.42
13	1.35	58	0.06	90	0.48
18	0.28	63	0.04	91	0.59
23	0.09	68	0.17	92	0.62
28	0.43	71	0.07	94	1.49
33	1.01	73	0.23	96	1.62
36	1.18	78	0.12	98	1.02
37	1.20	82	0.04		
38	1.47	84	0.08		

Tubes 0-18 were analysed to give the total base content and tubes 20-100 to give the weight of base in the upper phase. From tubes 0-20 was recovered a gum (356 mg.) which after neutral and phenolic material had been extracted reduced to a clear base (254 mg.). This was dissolved in dilute hydrochloric acid (10 ml.) and several drops of 40% hydrobromic acid added, when a crystalline hydrobromide was obtained m.p. 235-6° after recrystallisation from dilute hydrobromic acid. The hydrobromide did not depress the melting point of authentic emetine hydrobromide (m.p.248-250°)

A clear gum (567 mg.) was recovered from tubes 25-49. On addition of the solution of oxalic acid to a hot alcoholic solution of this gum a hydrogen oxalate crystallised 605 mg., m.p. 162-4° with prior sintering; O-methylpsychotrine hydrogen oxalate m.p. 150-162°.

Addition of oxalic acid solution to an alcoholic solution of the reddish-brown gum (30 mg.) recovered from tubes 82-91 was followed by crystallisation of a hydrogen oxalate (14 mg. m.p.165-6°; emetamine m.p. 165-172°).

Tubes 92-99 yielded a deep brown gum (104 mg.) from which a further quantity of hydrogen oxalate (14 mg. m.p. 163-5° was obtained. From the mother liquors a clear yellow base (33 mg.) was recovered but it formed no crystalline salts.

The bases from tubes 50-81 were not recovered.

These results are summarised in table 2.

Table 2. Recovery of bases from distribution at pH6.4
Wt. of base distributed - 1.26g.

Fraction	Wt. of base recovered mg.	Salts formed
0-20	356	hydrobromide
25-49	567	hydrogen oxalate (605 mg.)
82-91	30	hydrogen oxalate (14 mg.)
92-99	104	hydrogen oxalate (14 mg.)

Large scale recovery (and initial fractionation) of the alkaloids from the bismuth iodide complex

The bismuth iodide complex (120 g.) was agitated for 4 hrs. with 2N sodium hydroxide (750 ml.) and ether (1 litre). A crude separation of the aqueous and ether layers was effected after which the ether extract was filtered through a Buchner funnel (filtercel) which greatly facilitated the final separation of the aqueous and ether layers. The bismuth oxide suspension was thrice extracted with further volumes of sodium hydroxide (500 ml.) and ether (500 ml.) and the ether extracts were combined with those from a similar extraction of a further quantity of bismuth iodide complex (120 g.). On evaporation a clear brown gum

(47.2 g.) was obtained which exhibited an olive-green fluorescence.

The total base was distributed for eight transfers between 1 litre phases of ethyl acetate and aqueous buffer made from 0.5M KH_2PO_4 (5 vols.) and 0.5M K_2HPO_4 (3 vols.). The lower phase was transferred in each instance. Recovery of the bases yielded fractions as recorded in table 3.

Table 3.

Tube Nos.	Fraction	Wt. in g.
0-1	P	6.8
2-6	Q	22.8 *
7-8	S	13.2

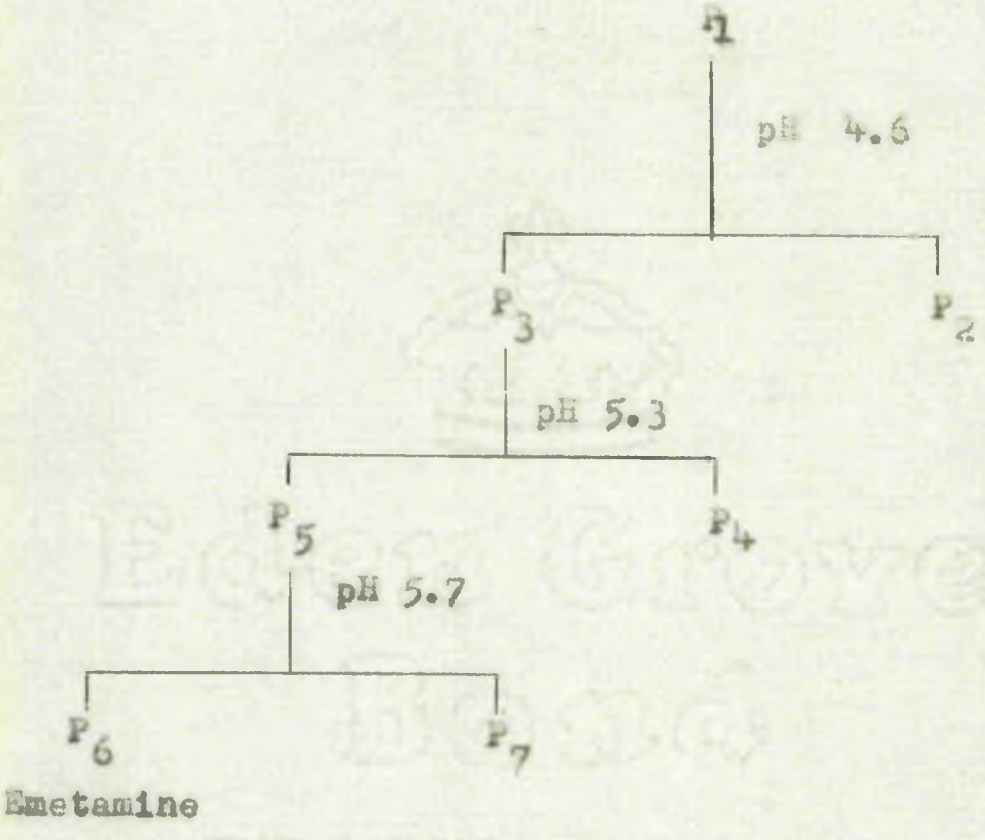
* Due to accidental losses from tubes 3 and 4 this figure is low.

Examination of Fraction P

The total base (6.8 g.) was suspended in ether and the solution filtered free from insoluble material. After treatment to remove phenolic material evaporation of the dried ether solution yielded a pale yellow base (4.4 g.: fraction P_1).

Fraction P_1 (1 g.) was scattered in five tubes and

Scheme 4. Fractionation of base P.



distributed for 95 transfers between ethyl acetate and aqueous phosphate buffer of pH4.6. Two distinct fractions were recovered corresponding to the peaks in Graph 2(a). Fraction P_3 (642 mg.) was recovered from tubes 0-30 and fraction P_2 from tubes 80-100. Fraction P_2 was reserved and later rejected whilst the material in fraction P_3 was scattered in three tubes and redistributed for 28 transfers between ethyl acetate and phosphate buffer solution of pH 5.3 when two fractions, P_4 (57 mg.) and P_5 (375 mg.) were again obtained corresponding to the analysis plotted in Graph 2(b). These fractions were recovered from tubes 25-31 and 0-16 respectively. The minor fraction was reserved and later rejected whilst fraction P_5 was redistributed at pH 5.7 for 33 transfers. As indicated by the plot of the analytical results in Graph 2(c) two fractions P_6 (217 mg.) and P_7 (31 mg.) were recovered from tubes 15-31 and 31-36 respectively.

The base from fraction P_6 was dissolved in ethanol (3 ml.) and a solution of oxalic acid in ethanol added when a hydrogen oxalate (171 mg.) crystallised: (recrystallised from ethanol m.p. 165-168°, $[\alpha]_D$ - 5.1°). Emetamine hydrogen oxalate has m.p. 165-172°, $[\alpha]_D$ = - 6.1°.

The hydrogen oxalates from the emetamine fraction were subsequently redistributed between ethyl acetate

and an aqueous phosphate buffer of pH 5.7 for 50 transfers when the distribution indicated in Graph 4 was obtained. The base (81 mg.) was recovered from tubes 29-36 and converted to the hydrogen oxalate (90 mg.).

Preparation of O-Methylpsychotrine hydrogen oxalate from fraction Q

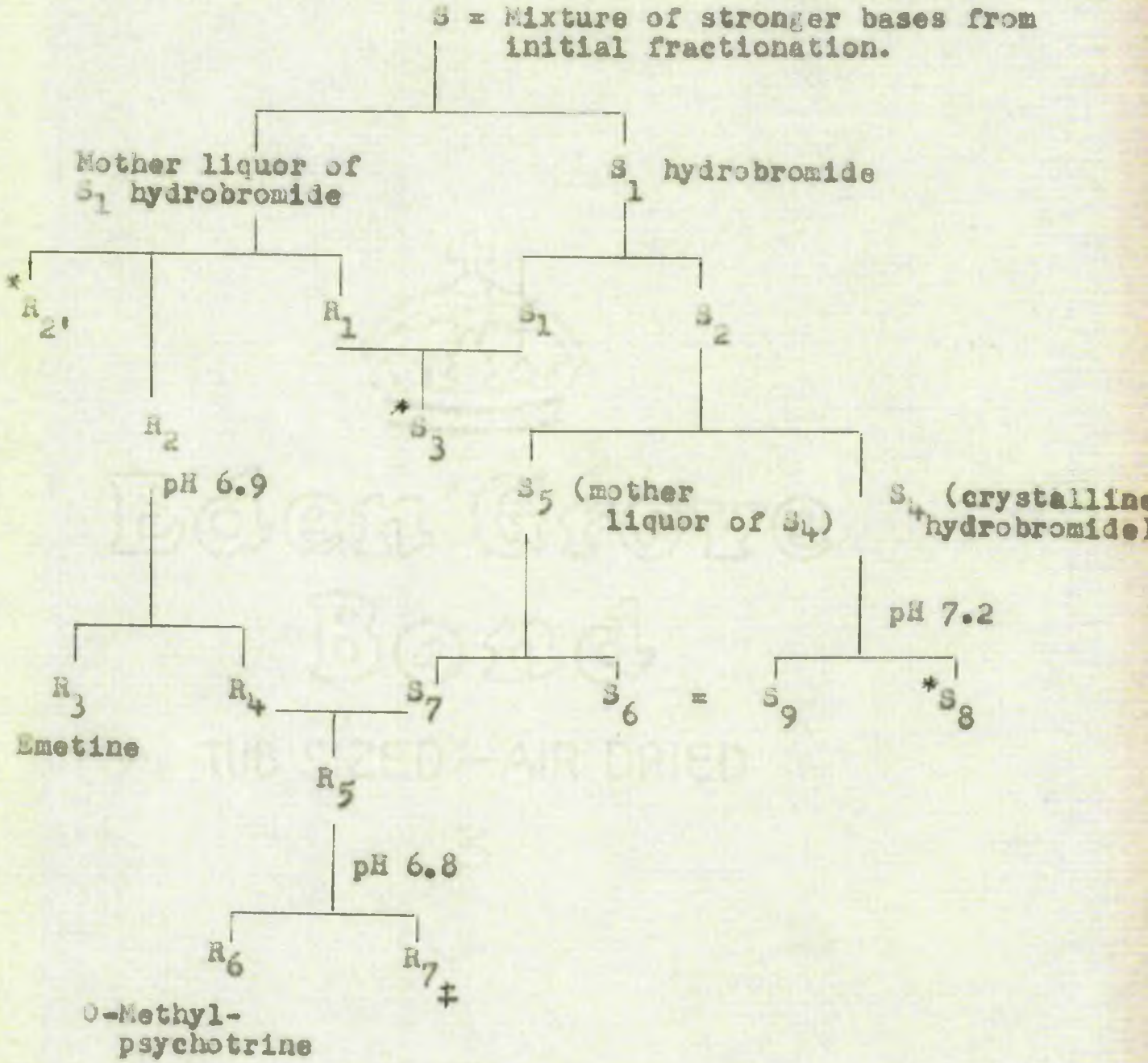
The base (22.8 g.) was dissolved in hot ethanol (300 ml.). On addition of an ethanolic solution of oxalic acid (12 g.) a hydrogen oxalate (25.7 g.) rapidly crystallised. Recrystallisation of the hydrogen oxalate (1 g.) from ethanol (500 ml.) yielded beautiful needles, (m.p. 162-3°, $[\alpha]_D$ + 43.3°). O-Methylpsychotrine oxalate m.p. 150-162° $[\alpha]_D$ + 45.9°

From the mother liquors of the crude hydrogen oxalate was recovered an ether-soluble base (3.1 g.) which gave no crystalline hydrobromide or hydrogen oxalate and was not studied further.

Examination of Fraction S

The clear red gum (13.2 g.) which had been obtained from the initial crude separation was dissolved in 2N hydrochloric acid solution and a solution of ammonium

Scheme 5 Fractionation of base S.



* Not examined in detail

‡ Gave no crystalline salts

bromide (12 g.) was added whereupon a thick brown gum separated which showed a tendency to crystallise.

The supernatant mother liquor was decanted off and on addition of alkali an ether soluble base (2.31 g.; fraction R_2) was extracted. On subsequent extraction of the aqueous alkaline phase with chloroform, fraction R_1 was obtained.

The gummy hydrobromide was dissolved in hot water and the solution made alkaline and extracted with ether when fraction S_2 (6.4 g.) was obtained. Extraction with chloroform gave fraction S_1 which was combined with fraction R_1. On evaporation of the chloroform a brown gum (1.7 g.) resulted. This was dissolved in ethanol and on addition of an excess of ether an insoluble material precipitated which was filtered off. A clear gum (659 mg.;S_3) was recovered from the alcoholic ether solution but it was not further examined.

Fraction R_2 was dissolved in dilute hydrochloric acid solution (30 ml.) and to the hot solution 40% hydrobromic acid (5 ml.) was added. The microcrystalline hydrobromide which crystallised slowly was collected and the base (567 mg.) was regenerated into ether. This base was dissolved in ethyl acetate, scattered in three tubes and distributed between ethyl acetate and aqueous phosphate buffer of pH 6.9. The results of the analysis after 56 transfers

are plotted in Graph 3(b). The base (300 mg.:R_3) recovered from tubes 10-35 was shown to be emetine hydrobromide whilst that from tubes 36-58 (96 mg.:R_4) was reserved. From the mother liquors of R_2 hydrobromide was recovered R_2

Fraction S_2 was reconverted to the hydrobromide (m.p.239-40°) and from this hydrobromide an ether soluble base (3.03 g.:S_4) was obtained. The mother liquors yielded a further quantity of base (3.4 g.:S_5).

Fraction S_4

The base S_4 (954 mg.) was scattered in five tubes and distributed between ethyl acetate and aqueous phosphate buffer solution of pH 7.2 when the distribution indicated in Graph 3(a) was obtained. From tubes 45-75 fraction S_9 (648 mg.) and from tubes 80-99 fraction S_8 (38 mg.) were recovered.

Fraction S_5

The base S_5 (998 mg.) was similarly distributed between ethyl acetate and aqueous phosphate buffer solution of pH 7.1 for 95 transfers after which fractions S_6 (349 mg.) and S_7 (519 mg.) were recovered from tubes 45-73 and 77-100 corresponding to the peaks in Graph 3(c).

Fraction S_7 was combined with fraction R_4 forming R_5 (620 mg.) which was distributed for 96 transfers between ethyl acetate and aqueous buffer of pH 6.8 after being

dissolved in tubes 0-3. The resultant distribution is plotted in Graph 3(d). Fractions R_6 (328 mg.) and R_7 (150 mg.) were obtained.

Identification of R_6

The base (328 mg.) was dissolved in a mixture of 40% aqueous hydrobromic acid (0.3 ml.) and water (2.5 ml.). On cooling feathery needles (98.4 mg.:m.p.197-210°) were precipitated. O-methylpsychotrine hydrobromide has m.p. 190-200°C. A base (288 mg.) was recovered from the mother liquors into ether which was evaporated to low bulk. Upon seeding with a crystal of O-methylpsychotrine the base crystallised (m.p. 123-4°, mixed m.p. with authentic O-methylpsychotrine 123-4°; O-me-psychotrine m.p. 123-4°). R_6 is therefore O-methylpsychotrine.

Identification of S_6

The base (349 mg.) gave a crystalline hydrobromide (201 mg.: m.p. 240-242°) from a solution in dilute hydrobromic acid. The hydrobromide in the mother liquor was precipitated by addition of sodium bromide as a gum which did not crystallise.

From the crystalline hydrobromide (48.6 mg.) was recovered the base (27.8 mg.). This was heated under reflux with benzoic anhydride (57.5 mg.) for 2 hours. The reaction mixture was then dissolved in ether and

extracted with dilute hydrochloric acid, from which extract the base was recovered into ether. Evaporation of the ether gave an oil which crystallised with difficulty to yield a crystalline base (28.4 mg.: recrystallised ethanol, m.p. 185-6°). N-benzoyl-emetine has m.p. 185-6° and as a mixed melting point gave no depression of this, S_6 was shown to be emetine.

Identification of S_9

S_9 (648 mg.) was dissolved in dilute hydrochloric acid and 40% hydrobromic acid solution added dropwise to the hot solution. Upon cooling a hydrobromide (861 mg.: recrystallised water m.p. 253-5°) was collected. Emetine hydrobromide has m.p. 250-60°. A portion of the base was converted as in the case of S_6 to the N.benzoyl derivative (recrystallised ethanol m.p. 184-5°).

As in admixture with authentic N-benzoyl emetine the melting point was not depressed S_9 is also emetine.

S_3, R_2, R_7 and R_8 gave no crystalline hydrobromides or hydrogen oxalates and were not examined further.

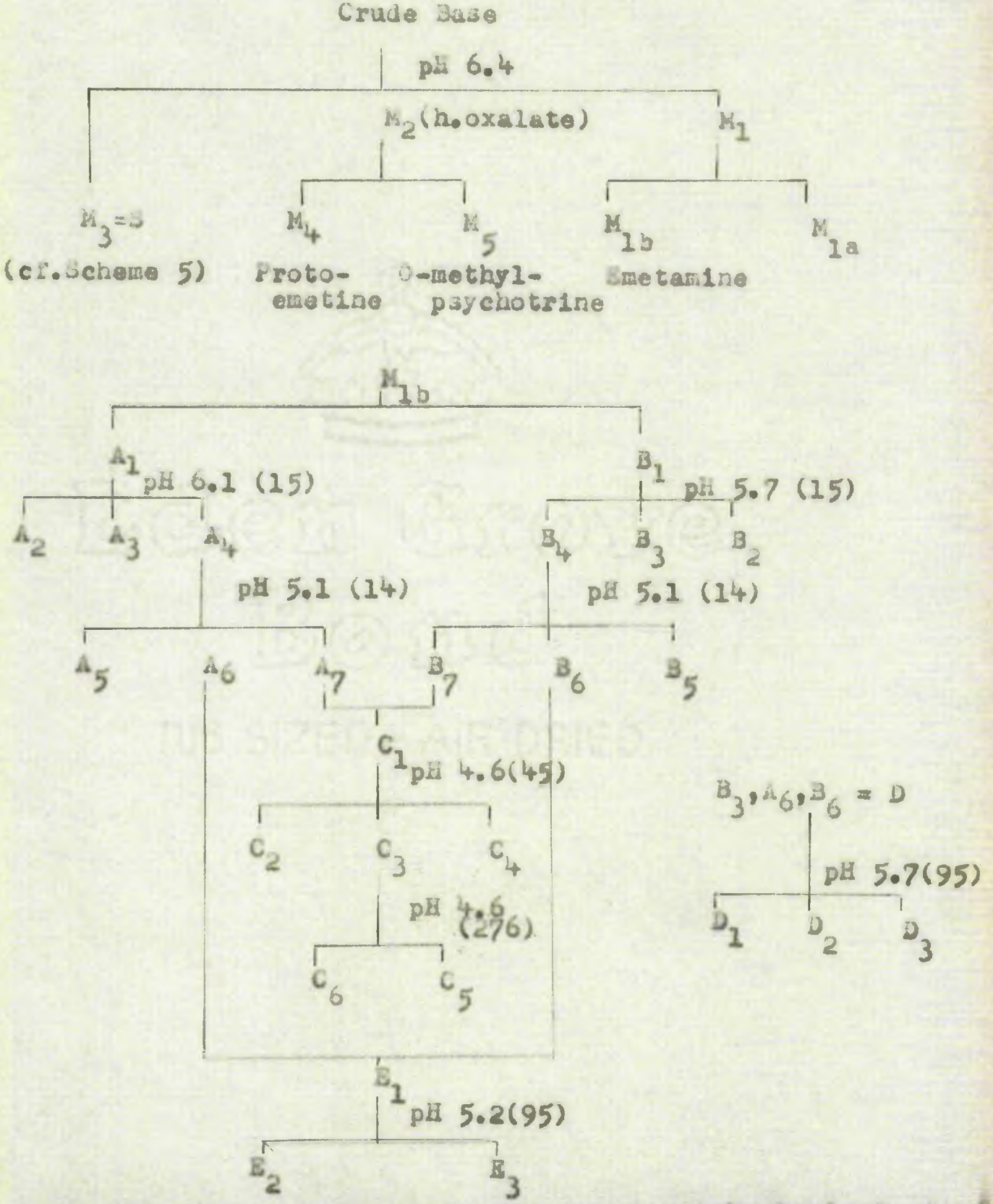
Examination of the mother liquors from the commercial extraction of emetine for emetamine and O-methylpsychotrine

The mother liquors from the crystallisation of emetine hydrobromide prepared from the bases extracted from Ipecacuanha root (900 lbs.) were basified by addition of aqueous ammonia and extracted exhaustively with chloroform. The chloroform was evaporated and the residue dissolved in hot 4% hydrobromic acid (770 ml.). On the solution being cooled a crystalline hydrobromide was obtained. The mother liquors of this hydrobromide were basified with aqueous ammonia, extracted into ethyl acetate (1000 ml.) and re-extracted into 2N hydrochloric acid (900 ml.). This acid solution was made alkaline by addition of aqueous ammonia and extracted into ether (1000 ml.). Evaporation of the ether gave a clear red gum (212 g.).

In three separate runs this base was distributed between 1 litre phases of ethyl acetate and 0.5M phosphate buffer solution of pH 6.4; the lower aqueous phases were transferred. The material was then divided into fractions M_1, M_2 and M_3 from tubes 0-1, 2-5, and 6-7 respectively. These fractions were then examined in detail(Table 6).

Fraction M_3 after basification with sodium hydroxide and extraction with ethyl acetate yielded, by evaporation of the solvent, emetine which was isolated as the hydro-

Scheme 6. Fractionation of the crude bases isolated from 900lbs. of Ipecacuanha root, after precipitation of emetine hydrobromide.



bromide and characterised as N-benzoyl emetine (m.p.184-5°)

The alkaline aqueous layer exhibited an intense green fluorescence which could be extracted into chloroform. The chloroform extract yielded an amorphous reddish-brown resin which was not further examined.

The upper layers from tubes 0-1 were each extracted twice with 0.5 M. potassium dihydrogen phosphate solution (1000 ml.), washed, dried and evaporated to leave a residue of weakly basic material (13.5 g. Mla). This was dissolved in 2N hydrochloric acid (200 ml.), insoluble material filtered off, the filtrate made alkaline with aqueous ammonia and extracted with ether (3 x 200 ml.). Evaporation of the ether solution gave a clear gum (0.607 g.) The insoluble material was digested with fresh dilute hydrochloric acid (250 ml.) and the acid extract worked up to give a further ether soluble fraction (0.46 g.). The two weakly basic fractions were combined and reserved. The buffer extracts (4 litres) from tubes 0-1 were basified by addition of strong potassium hydroxide solution and extracted with ethyl acetate which was evaporated to yield a clear reddish-brown gum (12.0 g. Mlb). This was dissolved in hot ethanol (100 ml.) and a solution of oxalic acid (9 g.) in ethanol (50 ml.) added. On concentrating the solution to 50 ml. a crystalline hydrogen oxalate (0.536 g.) separated overnight. Recrystallised

from ethanol the hydrogen oxalate had m.p. 170-172°; $[\alpha]_D$ = -5.8; emetamine hydrogen oxalate m.p. 171-3°; $[\alpha]_D$ = -6.1°.

From the mother liquors of the hydrogen oxalate a clear red gum (11.75 g.) was recovered and this was fractionated in two parts A_1 and B_1.

Examination of A_1

Fraction A_1 (5.25 g.) was scattered in tubes 0 and 1 and distributed for 14 transfers between 100 ml. phases of ethyl acetate and phosphate buffer (pH 6.1). Analysis showed no distinct peak and the material was recovered from tubes 0-6, 7-12 and 13-15 to give fractions A_2(0.42 g.), A_3(1.62 g.) and A_4(2.81 g.). None of these fractions crystallised.

Fraction A_3 was dissolved in ethanol (15 ml.) and an excess of oxalic acid added. The crystalline hydrogen oxalate (210 mg.) which was obtained was combined with that from fraction B_3 (q.v.)

Fraction A_4 was redistributed at pH 5.1 for 14 transfers. Analysis showed a peak at tube 8 and the bases were recovered from tubes 0-3, 4-10 and 11-14 to give fractions A_5(0.57 g.), A_6(1.18 g.) and A_7(0.68 g.).

Fraction A_5 yielded a hydrogen oxalate (23.4 mg.; m.p.185-7°; sint.169-70°) on addition of oxalic acid to

a solution of the base in ethanol. From the mother liquors stout prisms (46 mg. m.p.193-200°, sint.179-80°) were obtained but were not further examined.

Fraction A_6 was dissolved in ethanol (5 ml.) with heating and a solution of oxalic acid (200 mg.) in hot ethanol (4 ml.) added. After several hours a small amount of crystalline hydrogen oxalate (ca. 50 mg.) deposited and was recrystallised from ethanol (4 ml.) but not further investigated. The bases from the mother liquors were recovered and examined along with B_6 (q.v.).

Fraction A_7 was similarly combined with B_7 and examined later.

Examination of Fraction B_1

Fraction B_1(6.5 g.) was scattered in tubes 0-1 and distributed for 15 transfers at pH 5.7 between 100 ml. phases of ethyl acetate and phosphate buffer. The bases were recovered from tubes 0-3, 4-12 and 3-15 to yield fractions B_2(0.637 g.), B_3(2.88 g.) and B_4(2.1 g.). The bases from fractions B_2 and B_3 were converted to the hydrogen oxalate. Fraction B_2 yielded no crystalline hydrogen oxalate. That from fraction B_3 was combined with the hydrogen oxalate from A_3 and recrystallised from ethanol (200 ml.) when the salt had m.p. 170-171°. The base crystallised on recovery into ether and evaporation of the solvent, m.p.142-3° (emetamine etherate m.p.143-4°) Fraction B_4 was redistributed at pH 5.1 for 14 transfers

and the bases recovered from tubes 0-3, 4-10 and 11-14 to give fractions B_5 (0.192 g.), B_6 (0.706 g.) and B_7 (0.835 g.) respectively. None of these fractions gave a crystalline base.

Fraction B_5 gave a small amount (ca. 5 mg.) and B_6 a further quantity of hydrogen oxalate which was combined with that from A_6 and examined later.

The base from the mother liquors of B_6 hydrogen oxalate was recovered and combined with that from A_6 to form E_1, which was redistributed at pH 5.2 for 90 transfers. The total base (1.35 g.) was scattered in six tubes. Analysis showed the presence of peaks at tubes 41 (K - 0.72) and 60 (K - 1.73). The bases were recovered in two fractions from tubes 25-55 (E_2: 0.65 g.) and 55-80 (E_3:0.18 g.) but neither fraction gave either crystalline base or salts.

Fractions A_7 and B_7 were combined to give C_1 (1.52 g.) which was scattered in five tubes and distributed for 45 transfers at pH 4.6. Analysis showed the presence of peaks at tubes 23 (K - 0.5) and 47 (K - 44). The bases were recovered from tubes 0-6, 17-29 and 36-50 to give respectively fractions C_2(0.022 g.), C_3(0.423 g.) and C_4(0.421 g.). No fraction gave either a crystalline base, picrate, hydrogen oxalate, hydrochloride or per-

chlorate.

Fraction C_3 was redistributed for 276 transfers in the same system as above. Analysis showed the presence of peaks at tubes 42 (K - 0.25) and 67 (K - 0.32). The base was recovered from tubes 20-47 and 55-85 to yield C_4 (0.088 g.) and C_5(0.076 g.) by the following procedure. The phosphate buffer was made alkaline to phenolphthalein by addition of sodium hydroxide and the base extracted into ethyl acetate. The ethyl acetate was then extracted with dilute hydrochloric acid and the acid extract basified. Extraction with ether followed by evaporation gave the free base. Neither C_4 nor C_5 could be crystallised and they did not give crystalline salts. Attempts to seed with nor-coralydin and nor-coralydin perchlorate were unsuccessful.

The hydrogen oxalates from A_6 and B_6 were combined with the mother liquors of the hydrogen oxalate obtained from B_3 and the bases recovered to give D (2.82 g.). A portion (1.2 g.) of this fraction was scattered in five tubes and distributed for 95 transfers at pH 5.7. Analysis then showed peaks at tubes 25(K 0.36), 44 (K 0.78) and 61 (K 1.60). The base was recovered from tubes 15-30, 35-50 and 55-70 to afford fractions D_1(0.297 g.), D_2(0.304 g.) and D_3(0.189 g.) respectively. None of these fractions crystallised.

Isocac Alkaloid B_2.

Fraction B_2 was dissolved in the minimum of ethanol and converted into the hydrogen oxalate which was recrystallised from ethanol (60mg.;sint.176-7°:m.p.182-9°). After drying at 100° at 0.1 mm. over phosphorus pentoxide the hydrogen oxalate analysed for

C: 63.3,63.7; H: 7.3,6.9; OCH_3: 21.6%

$C_{30}H_{42}O_5N_2(COOH)_2$ requires C: 63.9; H: 7.4; OCH_3: 21.2%

Recovered from its oxalate the base subsequently crystallised from ether as colourless rosettes of needles (22 mg., m.p. 143-6°, unchanged by further recrystallisation from ether.

Found: C: 71.2; H: 8.2; N: 5.6%

$C_{30}H_{42}O_5N_2$ requires C: 70.3; H: 8.1; N: 5.6%

From the base in ethanolic solution was obtained a crystalline picrate which upon recrystallisation from aqueous acetone melted at 195-6°.

Found: C: 59.1; H: 5.9; N: 8.9%

$C_{30}H_{42}O_5N_2$ monopicrate requires C: 58.5; H: 6.1; N: 9.5%

Equivalent weights of 781 and 794 were determined for the picrate from the ultra-violet absorption using the method of Spring (58). [c=1.78mg./10ml.: $O.D._{357.5}$ = 0.362; $O.D._{380}$ = 0.295]

The calculations were based on $\epsilon_{357.5}$ = 16,140 and ϵ_{380} = 1295 for picric acid.

Assuming a molecular weight of 580 for the free base the ultraviolet absorption of the hydrogen oxalate determined in aqueous solution suggests the presence of two veratryl groups in the molecule

C - 1.07mg/100ml.

λ mμ	O.D.	
282.5	0.115	Maximum
254	0.0173	Minimum
227	0.304	Inflection

Attempted microhydrogenation of B_2 hydrogen oxalate

The hydrogen oxalate (9.2 mg.) was dissolved in water and hydrogenated over platinum. No hydrogen was absorbed over 2 hours. The catalyst was filtered off, the base recovered and converted into the hydrogen oxalate (m.p.186-8° (dec.) sint.173-4°). In admixture with the original base hydrogen oxalate the melting point was not depressed.

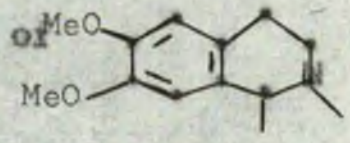
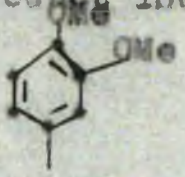
Mercuric acetate oxidation of alkaloid B_2

The non-crystalline alkaloid (7.1 mg.) recovered from the hydrogen oxalate was dissolved in a glacial acetic acid (0.2 ml.) and water (1 ml.). Mercuric acetate (70 mg.) was added and the solution heated under flux. The solution, initially colourless, immediately turned a greenish-yellow colour and mercurous acetate was precipitated. After boiling for 1½ hours the solution was filtered to remove the mercurous acetate and the filtrate was treated with

hydrogen sulphide, boiled, acidified, treated further with hydrogen sulphide and warmed to coagulate the mercuric sulphide. The solution was filtered and the filter-cake extracted with acetone until the final extract was colourless. The acetone extracts were combined with the main filtrate and evaporated to dryness. The ultraviolet absorption of the residue in aqueous solution was measured.

max. 245; 291; 302; 251 mu

min. 232; 5; 269; 297; 323 mu

A similar absorption spectrum would be obtained from the presence of a 6:7-dimethoxy-3:4-dihydroisoquinoline moiety and a veratryl absorption in the product indicating the presence of  and  in the original base.

Examination of Fraction M_2 - isolation of proto-emetine

Fraction M_2 (47 g.) was dissolved in ethanol (400 ml.) and an excess of oxalic acid dissolved in hot ethanol added. O-methylpsychotrine hydrogen oxalate (41 g.; m.p. 162-164°) crystallised rapidly. The base was recovered from the mother liquors as a gum (17.5 g.)

A portion (1.06 g.) of this base was scattered in five tubes and distributed for 95 transfers at pH 6.4. Analysis showed the presence of a peak at tube 63 (K - 1.80).

The base (402 mg.) was recovered from tubes 52-72. It did not crystallise from ether on seeding with O-methylpsychotrine and gave no sparingly soluble hydrogen oxalate. On addition of a slight excess of perchloric acid to a solution of the base in 50% aqueous ethanol a pale yellow crystalline perchlorate (421 mg.) was obtained. After recrystallisation from a mixture of water (15 ml.) and ethanol (5 ml.) (charcoal) the perchlorate had a melting point of 142-144° when heated from cold or 150-151° with prior sintering at 143° when inserted in a hot oil bath. These samples of the salt had been dried at room temperature over phosphorus pentoxide. Analysis of the salt dried further for 1½ hrs. at 100°C. gave C 52.4; H 5.2; OMe 14.8

The ultraviolet absorption of the base perchlorate

in ethanolic solution possessed a single maximum at 283 mu indicative of a veratryl absorption.

Attempted benzoylation of M_2

The alkaloid (95.6 mg.) was dissolved in ether (3 ml.) and benzoic anhydride (263 mg.) added. The ether was evaporated and the mixture heated on the steam bath in a stoppered flask for five hours. The reactants were then dissolved in dilute hydrochloric acid and ether. From the exhaustive ether extraction was obtained a neutral fraction (38 mg.) which on digestion with dilute acid gave benzoic acid and thus must have been mainly benzoic anhydride. The acid layer was made alkaline with sodium hydroxide and extracted with ether. Evaporation of the extracts yielded a basic fraction (56 mg.) which gave a crystalline perchlorate (m.p.150-151°; mixed m.p. with starting material 150-151°). The base must therefore be tertiary.

Microhydrogenation of M_2 perchlorate

The perchlorate (72.1 mg.) was dropped into ethanol (10 ml.) containing a suspension of platinum (ca. 10 mg.) in the hydrogenation apparatus. Hydrogen (1.07 ml.) was absorbed in the initial two hours and the reduction was stopped after 72 hours when the hydrogen uptake totalled 3.31 ml. (1.45×10^{-3} moles.) If it is assumed that one mole of hydrogen has been absorbed the molecular

weight of the perchlorate is 498.

The solution was filtered and concentrated to 3 ml. On cooling a crystalline perchlorate (38.6 mg.) was obtained which after recrystallisation from ethanol melted at 194-195°C. N-methylemetine perchlorate melts at 202-3° but a mixed melting point between the two salts depressed the melting point to 181-2°.

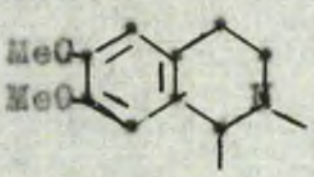
A comparison of the x-ray powder photographs of the N-methylemetine and the reduced alkaloid perchlorate proved the non-identity of the two compounds.

From the mother liquors of the reduced perchlorate was obtained a gummy, yellow perchlorate (18 mg.) which later crystallised (m.p.123-4°). A third crop of crystals consisting of a white salt (4 mg; m.p.153-4°) was also obtained.

Mercuric acetate oxidation of M_2

The oxidation was carried out and the products worked up as in the oxidation of alkaloid D_3. In this case the base (25 mg.) mercuric acetate (200 mg.), acetic acid (0.5 ml.), several crystals of sodium acetate (hydrated) and water (3-4 ml.) were heated under reflux for 5½ hrs. There was no increase in colour from the original pale yellow but mercurous acetate was formed which indicated that oxidation had taken place.

The base was recovered as a green gum (21.0 mg.) which could not be crystallised and formed neither hydrogen oxalate nor perchlorate. An ultraviolet absorption analysis of a portion of this gum dissolved in ethanol showed maxima at 246.5, 303 and 351.5 mu indicative of the structure



in the original compound.

Equivalent weight of the alkaloid M_2

Alkaloid M_2 perchlorate was dried at 80° for 1½ hours over phosphorus pentoxide at 0.05 mm. A portion (5.80 mg.) of this in 1:1 aqueous ethanol (5 ml.) was titrated with 0.00976 N sodium hydroxide using phenolphthalein when 1.18 ml. of alkali were consumed, which led to an equivalent weight of 503 for the salt. A repeat determination gave a value of 498.

Synthesis of Model Compounds

Cyclohexene acetonitrile

Cyanoacetic acid (50 g.) dissolved in piperidine (68 ml.) was reacted with cyclohexanone (60 g.) at room temperature for 1½ hrs. and subsequently heated on a steam bath for a similar period when a vigorous evolution of carbon dioxide was observed. The reaction mixture was cooled, acidified by addition of 6N hydrochloric acid and extracted with ether (2 x 250 ml.). The product, upon evaporation of the ether extract, was distilled under reduced pressure (9mm Hg) and the following fractions collected:

(i) 15.94 g. distilling at 40-45°

(ii) 4.24 g. distilling at 70-72°

(iii) 30.0 g. distilling at 85-90° (49.5%)

Cyclohexane acetonitrile

Cyclohexene acetonitrile (29.8 g.) dissolved in ethanol (100 ml.) was hydrogenated in the presence of 10% palladium on strontium carbonate (1.63 g.) as catalyst. Absorption of hydrogen ceased after 5.56 litres had been absorbed (1 mol. H_2 requires 5.59 litres). Filtered free from catalyst the solvent was removed under reduced pressure. The nitrile was dissolved in ether (100 ml.) and the solution was extracted with dilute hydrochloric acid, washed with water and dried over sodium sulphate before

the nitrile was recovered by evaporation of the ether.

Cyclohexane acetic acid

Concentrated sulphuric acid (45 ml.) was added to the nitrile (29.5 g.) with cooling and the solution left for 15 hrs. in an open flask after which water (90 ml.) was added and the mixture was heated under reflux for a total of 17 hrs. during which time a further 60 ml. of sulphuric acid and water (90 ml.) were added when the reaction mixture darkened considerably. The solution was then cooled, diluted with water (100 ml.) and extracted with equal volumes of ether and ethyl acetate. The combined extracts were extracted with dilute sodium hydroxide (3 x 50 ml.). Acidification of the alkaline extracts gave a dark coloured acid (21.2 g.) which was extracted into ethyl acetate. On evaporation of the solvent the acid was obtained as a colourless liquid, distilling under 9 mm Hg pressure at 140-145° (bath temperature), which crystallised in rosettes of needles (m.p.30-35°) and yielded an amide m.p.168-169°. Cyclohexane acetamide m.p. 168°.

Cyclohexane acetyl 2-(3:4-dimethoxyphenyl) ethylamide

Cyclohexane acetic acid (5.00 g.) was heated under reflux for 70 minutes with thionyl chloride (15 ml.) when the excess thionyl chloride was distilled off and the acid chloride dissolved in dry ether (25 ml.). This solution was added dropwise with vigorous stirring to a solution

of β-3:4-dimethoxyphenylethylamine (12.8 g.) in dry ether (100 ml.). After shaking thoroughly for 10 minutes water was added to dissolve the precipitated hydrochloride and the amide was extracted into ethyl acetate which in turn was extracted with dilute hydrochloric acid, sodium hydroxide, washed with water and dried. The crude amide (14.7 g.) obtained upon removal of the solvent recrystallised from toluene in plates, 7.0 g. (67%) m.p.106-7°.

Analysed	C: 71.1	H: 8.1	N: 4.9
$C_{18}H_{27}O_3N$ requires	C:70.80	H:8.9	N:4.6%

1-hexahydrobenzyl-3:4-dihydro-6:7-dimethoxyisoquinoline

The amide (4.87 g.) was dissolved with warming in dry toluene (15 ml.) and the solution heated under reflux for 1 hr. with phosphorous oxychloride (6.5 ml.) at a bath temperature of 130°. Water (30.ml.) was added and the toluene layer was then extracted with dilute hydrochloric acid (3 x 30 ml.). The combined aqueous extracts were washed with ether, made alkaline with sodium hydroxide and extracted with ether (3 x 100 ml.). Evaporation of the dried extract gave the dihydroisoquinoline (4.7 g.; 98%) which formed a picrate, m.p.204-205°, and a perchlorate, m.p.153-155°. The latter was recrystallised from ethanol and dried at 110° and 0.05 mm.Hg over phosphoric oxide.

Found	C:55.7	H:6.9	N:4.3
$C_{18}H_{26}NO_6Cl$ requires	C:56.02	H:6.75	N:3.63%

The free base, recovered from the perchlorate as a colourless glass, crystallised in rosettes of needles, m.p.75-76°, which sublimed at 130-140° and 0.1 mm.Hg pressure.

Found	C:75.2	H:8.6	N:5.2
$C_{18}H_{25}O_2N$ requires	C:75.2	H:8.77	N:4.87%

Preparation of substance M

Sulphuric acid (25 ml.) was added cautiously with cooling to cyclohexene acetonitrile (18.4 g.) prepared as previously described, and digested at room temperature for 16 hrs. Water (50 ml.) was added slowly with cooling and the solution heated under reflux for 6½ hrs. when more water was added and the heating was continued for a further 8 hrs. After standing overnight the acid solution was extracted several times with ether. The ether extract (600 ml.) was extracted with dilute sodium hydroxide solution. On reacidification an unsaturated acidic fraction, m.p.52-56°, was extracted into ether solution. The acid distilled at 136-142° under 10 mm.Hg pressure to yield a colourless solid m.p.66-72° (Cyclohex-1-enyl-acetic acid, m.p.13°. Cyclohexylideneacetic acid m.p.92°).

The acid (3.5 g.) was dissolved in a mixture of ethanol (30 ml.) and glacial acetic acid (10 ml.) and hydrogenated over platinic oxide. Due to a leak in the

apparatus the hydrogen uptake could not be determined but the product (3.3 g.;m.p.29-33°) did not decolourise potassium permanganate solution. Cyclohexyl acetic acid has m.p.33°.

The reduced acid (3.3 g.) was converted to the acid chloride by treatment with thionyl chloride (6 ml.). A dry ethereal solution of the acid chloride was then added with stirring to a solution of β-3:4-dimethoxyphenyl-ethylamine (5.6 g.) in dry ether. The reaction was worked up as before and the amide (5 g., m.p.94-96°) recrystallised from toluene. When dried over phosphoric oxide at 65° in vacuo it had m.p.95.5-96°.

Found	C:71.1	H:8.1	N:4.9
$C_{18}H_{27}O_3N$ requires	C:70.8	H:8.9	N:4.6

A portion of the amide (2 g.) was cyclised with phosphoryl chloride (4 ml.) in dry toluene in the usual manner and the basic product (1.955 g.) was dissolved in ethanol. Excess oxalic acid was added when the hydrogen oxalate of substance M (375 mg.) crystallised; the mother liquor was retained. Several times recrystallised from ethanol the salt had m.p.175-6° (air dried). When dried at 110° for 2½ hrs. in vacuo over phosphoric oxide the hydrogen oxalate exhibited a transition point at 164-165° and melted at 182-183° with prior sintering at 179°.

Found	C:64.4	H:5.80	N:3.7
	64.4	5.74	3.78

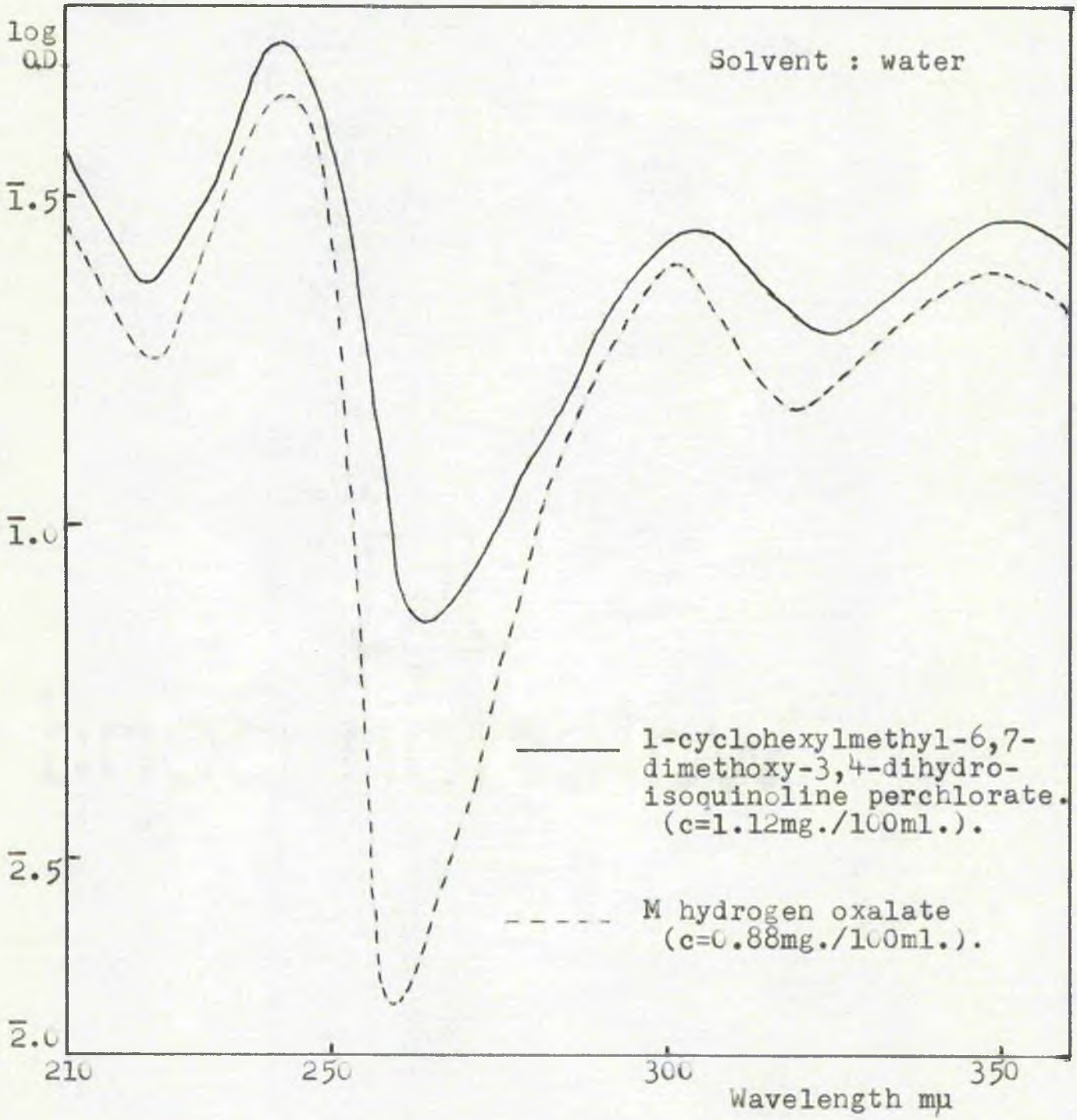
The compound contains no C-methyl group.

From the mother liquor of the hydrogen oxalate the base was recovered and converted to the picrate (1.41 g. m.p.166-172°) which on recrystallisation from ethanol was raised to 173.5-175.5°. The base recovered from the picrate formed a crystalline perchlorate which after recrystallisation from ethanol had m.p.142-3°. The mixed m.p. with authentic 1-hexahydrobenzyl-3:4-dihydro-6:7-dimethoxyisoquinoline perchlorate (m.p.154-5°) was 147-9°.

Hydrogenation of the hydrogen oxalate

A solution of the hydrogen oxalate (26.5 mg.), previously dried at 110° over phosphoric oxide in vacuo, was dissolved in ethanol (5 ml.) and hydrogenated at atmospheric temperature and pressure in the presence of platinic oxide (10 mg.). Absorption of hydrogen (0.94 mole) ceased after 20 minutes. The hydrogen oxalate of the reduced compound was insoluble in ethanol but when dissolved in water absorbed no further hydrogen.

Ultraviolet absorption spectra of 1-cyclohexylmethyl-6,7-dimethoxy-3,4-dihydroisoquinoline perchlorate and Substance M hydrogen oxalate.



Phenylacetyl-2-(3:4-dimethoxyphenyl)-ethyl amide.

Phenylacetic acid (1·00 g.) was converted to phenylacetyl chloride by reaction with thionyl chloride. The crude product obtained on removal of the excess thionyl chloride was treated directly with a solution of 3:4-dimethoxy β-phenylethylamine (3·1 g.) in ether. The crude phenylacetyl-2-(3:4-dimethoxyphenyl)-ethylamide (from this reaction mixture) recrystallised from benzene in needles, m.p.158-9°.

1-benzyl-3:4-dihydro-6:7-dimethoxyisoquinoline.

The amide was cyclised by heating under reflux with phosphorus oxychloride (5 ml.) in boiling toluene (10 ml.) for 1 hour. The reaction mixture was then cooled and water (30 ml.) added. The toluene layer was diluted with ether (20 ml.) and the aqueous layer extracted with dilute hydrochloric acid (3 x 30 ml.). The combined aqueous extracts were washed with ether, made alkaline by addition of ammonium hydroxide solution and extracted with ether (3 x 30 ml.). Evaporation of the ether gave the dihydroisoquinoline (0·8 g.) which crystallised in needles and was recrystallised from petroleum ether, m.p.101-2°.

The dihydroisoquinoline formed a hydrogen oxalate which was recrystallised from ethanol when it sublimed from 255° and melted at 263-4°.

1-Methyl-6:7-dimethoxy isoquinoline

1-Methyl-6:7-dimethoxy isoquinoline was prepared in 38% yield by the method of Späth and Polgar (78) and melted at 108-108.5°. The picrate had melting point 245-6° (S. & P. 247-9°).

β-3:4-dimethoxyphenylethylamine (9.0 g.) was converted to its acetyl derivative (5.5 g.;50%). Cyclisation with phosphoric oxide in boiling toluene afforded the dihydroisoquinoline (3.76 g.;74%) which was dehydrogenated with 20% palladium on charcoal to 1-methyl-6:7-dimethoxy isoquinoline (2.67 g.;71%).

1-Styryl-6:7-dimethoxy isoquinoline (75)

1-Methyl-6:7-dimethoxy isoquinoline (1.02 g.) was heated under reflux with benzaldehyde (8.2 ml.) in the presence of zinc chloride (0.62 g.) for 5 hrs. at a bath temperature of 170-180°. The reaction mixture was diluted with benzene (30 ml.) and dilute hydrochloric acid added. The sparingly soluble hydrochloride (m.p.204-205°) was collected, suspended in dilute ammonia and the base extracted into ethyl acetate. Evaporation of the dried extract gave 1-styryl-6:7-dimethoxyisoquinoline, m.p.167-167.5° (air dried). The melting point was not raised by recrystallisation from ethanol.

Found	C:76.6	H:5.80	
$C_{19}H_{17}O_2N$ requires	C:78.34	H:5.89	N:4.8

6:7-dimethoxyisoquinaldic acid

1-styryl-6:7-dimethoxy isoquinoline (750 mg.) was dissolved in 50% aqueous acetone (65 ml.). A solution of potassium permanganate (1.312 g. 3 equivs.) in water (75 ml.) was added over 30 minutes with stirring and the temperature maintained at 0-5°. The solution was then stirred for 1 hr. whilst the temperature was allowed to rise to room temperature. After the solution had been filtered and the pad extracted with boiling aqueous acetone the filtrate was concentrated to 40 ml. and washed with ethyl acetate (2 x 25 ml.). The pH of the aqueous solution was adjusted to pH4-5 with dilute hydrochloric acid and the solution kept overnight at 4°C. Orange-brown needles (453 mg.;75%) crystallised and were collected, m.p.204-205° (with decarboxylation). [Pyman quotes m.p.208°(corr.) for 6:7-dimethoxy isoquinaldic acid.]

The needles were soluble in dilute ammonium hydroxide and dilute acid and gave a deep orange colour with ferrous sulphate solution.

The needles (15 mg.) were heated at 220° until evolution of carbon dioxide had ceased. The resulting liquid gave a picrate which recrystallised from ethanol had melting point 228-229°C.(Pyman quotes 227° for 6:7-dimethoxy isoquinoline).

Oxidation of model compounds

1. 1-Methyl-6:7-dimethoxy isoquinoline

1-Methyl-6:7-dimethoxy isoquinoline (510 mg.) was dissolved in water (25 ml.). A solution of potassium permanganate (397 mg.;3 equivalents of oxygen) in water (40 ml.) was added dropwise with stirring and the temperature maintained at 50°. The solution had decolourised after 1½ hrs. when the manganese dioxide was filtered off, the filter pad extracted with acetone and the filtrate concentrated to remove the acetone. The concentrate was brought alkaline to phenolphthalein by addition of 2N sodium hydroxide solution, extracted with ether, brought to pH5-6 by addition of dilute hydrochloric acid and extracted with chloroform. The residue from the ether and chloroform extracts (285 mg.) crystallised from ether and gave a picrate, m.p.245-6° both alone and in admixture with 1-methyl-6:7-dimethoxy isoquinoline picrate.

The aqueous solution was evaporated to dryness and dissolved in 6 ml. of water. No crystals of 6:7-dimethoxy isoquinaldic acid could be obtained at pH 5 even upon seeding the solution with an authentic sample.

2. 1-hexahydrobenzyl-3:4-dihydro-6:7-dimethoxy isoquinoline

A solution of potassium permanganate (220 mg.) in water (25 ml.) was prepared. The base (200 mg.) was dissolved in 1:1 aqueous acetone (50 ml.). This solution was stable to

five drops of the permanganate solution over four hours at room temperature.

3. 1-hexahydrobenzyl-2-benzoyl-3:4-dihydro-6:7-dimethoxy-isoquinoline - preparation of.

The base (1.30 g.) was heated with benzoic anhydride (2.01 g.) in a stoppered flask on the steam bath for 4 hrs.. The contents were dissolved in ethyl acetate which was extracted in turn with 2N hydrochloric acid and 2N sodium hydroxide. The residue obtained upon evaporation of the dried ethyl acetate extract was recrystallised from ethanol (0.854 g.;45%) m.p.186-9°.

4. Oxidation of 1-hexahydrobenzyl-2-benzoyl-3:4-dihydro-6:7-dimethoxyisoquinoline.

A solution of 1-hexahydrobenzyl-2-benzoyl-3:4-dihydro-6:7-dimethoxyisoquinoline (80 mg.) in acetone (120 ml.) was not decolourised when heated under reflux for 30 minutes with five drops of a solution containing 2% potassium permanganate. Saturated sodium carbonate solution (4 ml.) was added but the solution remained stable when heated for a further hour, when the starting material was recovered. Under similar conditions N-benzoyl emetine was rapidly oxidised by potassium permanganate.

1-Hexahydrobenzyl-2-benzyl-3:4-dihydro-6:7-dimethoxy isoquinolinium chloride

The base (2.49 g.) was heated with benzyl chloride (10 ml.) in a stoppered flask at 100-120° for 6½ hrs. On cooling the quaternary salt (2.41 g.) crystallised out from the reaction mixture, was filtered off, washed with ether and acetone and dried in a desiccator. Concentration of the benzyl chloride and acetone filtrates yielded a second crop. The isoquinolinium chloride was recrystallised from acetone when it melted at 180-190° with decomposition.

1-Hexahydrobenzyl-2-benzyl-6:7-dimethoxy-3:4-dihydro-isoquinolinium iodide

Excess potassium iodide solution was added to the isoquinolinium chloride dissolved in aqueous acetone. The solution was warmed to dissolve the precipitated iodide and on being cooled the iodide crystallised in plates. Recrystallised from ethanol the iodide, m.p.208-209°, was dried at 100° for three hrs. over phosphoric oxide in a vacuum and submitted for analysis.

Found	C:59.9	H:6.3	N:2.8
$C_{25}H_{32}O_2NI$ requires	C:59.4	H:6.38	N:2.77

Oxidation of 1-Hexahydrobenzyl-2-benzyl-3:4-dihydro-6:7-dimethoxy isoquinolinium chloride with potassium permanganate

(i) in aqueous acetone

The isoquinolinium chloride (1.00 g.) was dissolved in 1:1 aqueous acetone (20 ml.) to which 2N sodium hydroxide

(1.5 ml.) was added. With the temperature maintained at 0-3°C, an aqueous solution (50 ml.) of potassium permanganate (839 mg.) was added dropwise over 20 minutes when the solution was completely decolourised. During the addition base began to be precipitated and more acetone (30 ml.) was added. The manganese dioxide was coagulated by heating on the steam bath. After the solution had been filtered and the pad washed well with acetone, the clear filtrate was concentrated to remove the acetone. The brownish-yellowish oil, which separated, was extracted with ethyl acetate. The aqueous layer was acidified with dilute hydrochloric acid and extracted with ethyl acetate (3 x 50 ml.). The combined ethyl acetate extracts were washed with dilute acid and extracted with saturated sodium carbonate solution (3 x 40 ml.). The ethyl acetate extracts upon evaporation afforded neutral material (676 mg.) which recrystallised from ether as needles (264 mg;37%)m.p.102-103° after recrystallisation from aqueous ethanol and being dried at 78° over phosphoric oxide in a vacuum.

Found	C:72.9	H:6.7	N:4.7
$C_{18}H_{19}O_3N$ requires	C:72.7	H:6.44	N:4.7

(ii) in aqueous dioxan

A study of the relative stabilities in the presence of alkali of acetone distilled from potassium permanganate and dioxan distilled from sodium showed that the acetone was appreciably attacked by permanganate as the pH rose to pH 8-9 whilst the dioxan remained stable after the addition of five drops of 2% permanganate solution.

To an aqueous solution (8 ml.) of the isoquinolinium chloride (1.00 g.) was added 2N sodium hydroxide (1.5 ml.) and dioxan (30 ml.) to give a homogeneous solution. Potassium permanganate (1.00 g.) was dissolved in water (60 ml.) and added dropwise with vigorous stirring and with the temperature maintained between 0-5°. As the colour of permanganate persisted after the addition of 33 ml. of the solution the addition was stopped. Two drops of ethanol destroyed the excess permanganate. When the reaction was worked up as before the yield of crystalline neutral material (349 mg.) was 48.8%. The acidic fraction (179 mg.;47%) which crystallised was low melting and smelt strongly of cyclohexyl carboxylic acid but was not characterised.

<u>Hydrogenolysis of 1-hexahydrobenzyl-2-benzyl-3;4 dihydro-6;7-dimethoxy isoquinolinium iodide</u>

10% palladium on charcoal (211 mg.) was suspended in 50% aqueous ethanol and saturated with hydrogen. When the 2-benzyl iodide (505 mg.) was dropped into the

suspension no uptake of hydrogen was observed over 1½ hrs. Sodium acetate (410 mg.) was added to the solution but again no uptake of hydrogen was observed.

Filtered free from the catalyst the solution was shaken for 2 hrs. with freshly precipitated silver chloride (1 g.). The silver salts were filtered off. The clear pale yellow filtrate was added to an aqueous suspension of palladium on charcoal (200 mg.) which had been equilibrated with hydrogen. Absorption of hydrogen (45.5 ml.; 1.9 moles at 753 mm./21°C) had ceased after 45 minutes.

The filtered solution from the hydrogenation was concentrated to remove the ethanol, made alkaline with dilute sodium hydroxide and extracted into ether (3 x 70 ml.). Evaporation of the dried extract gave a base (215 mg.; 74.4%) which yielded quantitatively a hydrogen oxalate, needles from ethanol, m.p.194.5-195.5°(dec.)

Found	C:63.0	H:7.7	N:3.7
$C_{20}H_{29}O_6N$ requires	C:63.3	H:7.7	N:3.8

Papaverine Benzochloride

Papaverine (990 mg.) was dissolved in benzyl chloride (5 ml.) and the solution heated on the steam bath for 1 hr., kept for 15 hrs. at room temperature and then concentrated under reduced pressure to remove the excess of benzyl chloride. The residue crystallised from 1:1 ethanol-

ethyl acetate to give 450 mg. of crystalline solid, m.p. 140-142° C. This was boiled with 350 ml. of ether (volume required to dissolve 450 mg. of papaverine is 260 ml.) and the insoluble material was collected to afford 302 mg. (22%) of papaverine benzochloride, m.p.152-153° (dec.)

Oxidation of Papaverine Benzochloride

Papaverine benzochloride (300 mg.) was dissolved in water (5 ml.) to which 2N sodium hydroxide (0.5 ml.) and dioxan (4.5 ml.) distilled from sodium, were added. The solution was cooled in an ice bath and an aqueous solution (35 ml.) of potassium permanganate (250 mg.) was added dropwise with stirring. The colour of permanganate which persisted after the addition of 14.4 ml. was destroyed by addition of hydrogen peroxide and the manganese dioxide was coagulated by heating on the steam bath. After the solution had been filtered and the pad washed well with acetone, the clear filtrate was concentrated to remove the organic solvents when 1-keto-2-benzyl-6:7-dimethoxy-1:2-dihydro-isoquinoline crystallised as feathery needles, (222 mg.) m.p.160-161°: the mother liquor was reserved. A sample for analysis was purified by sublimation of the solid at 150-160° (bath)

(0.06 mm.) and then had m.p.162-163° (lit.167°) (79)

Analysis	C: 72.6	H: 5.70	N: 4.8
$C_{18}H_{17}O_3N$ requires	C: 73.2	H: 5.80	N: 4.74

The mother liquor above was concentrated to 20 ml., acidified with dilute hydrochloric acid and extracted thrice with ethyl acetate (20 ml.). The combined extracts were washed thrice with 2N sodium hydroxide (10 ml.), then with water and finally dried over sodium sulphate. Evaporation of the solvent left a neutral fraction (58 mg.) which gave a crystalline 2:4-dinitrophenylhydrazone. Recrystallised from ethyl acetate this formed red needles, m.p.264-264.5°C alone and in admixture with the pure 2:4-dinitrophenylhydrazone of veratraldehyde.

In a control experiment veratraldehyde (5 mg.) in water (1 ml.) and dioxan (3 ml.) at 0°C only decolourised permanganate very slowly. Acetaldehyde under the same conditions was rapidly oxidised by potassium permanganate.

Preparation and oxidation of emetamine dibenzochloride

A solution of emetamine (86 mg.) in benzyl chloride (2 ml.) was heated on the steam bath for 3 hrs., kept overnight and heated for a further half hour before distilling off the majority of the benzyl chloride under reduced pressure. The benzochloride crystallised when heated with ether (5 ml.) and was washed thoroughly with a second portion

of ether (25 ml.). The quaternary chloride (121 mg.; 87% as dibenzochloride) was collected on a hardened filter paper, washed again with ether (20 ml.) and dried over phosphorous pentoxide and silica gel in vacuo.

When transferred to a 100 ml. conical flask with water (4 ml.) the quaternary salt crystallised in fine plates but dissolved on addition of 2N sodium hydroxide (0.15 ml.) and dioxan (16 ml.). With the flask immersed in an ice bath, potassium permanganate (56 mg.; 3.3×10^{-3} equivs.) in water (5 ml.) was added dropwise to the solution with vigorous stirring. When the addition was complete no permanganate colour persisted and the manganese dioxide was coagulated by heating on the steam bath.

The solution was worked up as in the papaverine case and feathery needles were obtained again when the organic solvents were evaporated. These were collected on a hardened filter paper (Whatman No.50) and dried over phosphoric oxide in vacuo (26 mg.; 48%). Sublimation at 145-150° (bath temp.) /0.1 mm. gave colourless needles, m.p.159-161°; mixed m.p. with 1-keto-2-benzyl-6:7-dimethoxy-1:2-dihydro-isoquinoline, 160-161.5°. The ultraviolet absorption spectra of the two products were also identical.

λ max.	249.5	282.5	294	312	322.5	336
log. ϵ max. (ex emetamine)	4.670	3.860	3.940	3.586	3.643	3.520
log. ϵ max. (authentic)	4.676	3.890	3.950	3.589	3.652	3.530

O-Methylpsychotrine dibenzochloride: Preparation & oxidation of.

O-Methylpsychotrine (10 g.) dissolved in benzyl chloride (100 ml.) was heated on the steam bath in a darkened, stoppered flask for 10½ hrs. The solution was concentrated under reduced pressure to 30 ml., cooled and poured into ether (600 ml.) when the amorphous benzochloride precipitated. The precipitate (19.4 g.) was washed with ether (200 ml.),suspended in ether (200 ml.) and filtered to afford a solid which clung tenaciously to benzyl chloride even after long drying in a vacuum desiccator. This salt could not be crystallised from acetone, methanol, ethanol or acetone-ether.

To a vigorously stirred solution of the benzochloride (5 g.) in water (50 ml.) and purified dioxan (150 ml.) was added at 4° sodium hydroxide (8 g.) in water (30 ml.). This was followed by potassium permanganate (4.55 g.) in water (230 ml.) added over fifteen minutes with vigorous stirring throughout. During the course of the oxidation the temperature of the reaction mixture rose by 8°. The final green solution was decolourised by coagulating the colloidal

manganese dioxide which was then filtered off and the filter pad extracted with boiling aqueous acetone.

This oxidation was repeated thrice on the same scale. All four filtrates were combined and concentrated to 500 ml. to remove acetone and dioxan. The brown oil which precipitated was extracted with ethyl acetate (2 x 600 ml.) and chloroform (5 x 200 ml.) and these extracts were reserved. The aqueous solution was then acidified with concentrated hydrochloric acid, extracted with ether (2 x 100 ml.) and was concentrated under reduced pressure to a volume of 100 ml. Ethanol (400 ml.) was added to precipitate inorganic salts which were filtered off and washed with ethanol (150 ml.). To the filtrate, concentrated to 50 ml., ethanol (200 ml.) was added and the inorganic salts were again removed by filtration and washed thoroughly. The final filtrate was reduced in bulk to 120 ml. and hydrogenated in presence of 10% palladium charcoal (524 mg.). Absorption of hydrogen (228 ml. at 752 mm./17°) ceased after 2 hrs.

The filtered solution from the hydrogenation was evaporated to dryness and after being dried overnight in a desiccator the residue was extracted with magnesium dried ethanol (3 x 100 ml.). The ethanolic extract yielded a gum which was esterified by being heated with absolute methanol (500 ml.) and concentrated sulphuric acid (21 ml.) under

reflux for 16 hrs. The solution reduced in volume to 100 ml. was then poured with stirring into a solution of potassium carbonate (75 g.) in water (100 ml.) containing crushed ice (100 g.). The amino ester was extracted with ether (4 x 300 ml.) and was recovered from the dried extract as a viscous base (2.97 g.)

The chloroform extract, above, yielded quaternary material (5.2 g.) which was reoxidised and worked up as above to afford a further portion of amino ester (589 mg.) which was combined with the main product and distilled at 150-160° (bath) and 0.2 mm. pressure to give the amino ester as a clear gum (2.874 g.).

The ethyl acetate extract yielded neutral material (5.96 g.) which was separated into an ether soluble and an ether insoluble fraction. The former crystallised from ether when seeded with 1-keto-2-benzyl-6:7-dimethoxy-1:2:3:4-tetrahydro-isoquinoline to afford needles (3.93 g.). These were recrystallised from ethanol to give a sample, m.p.85-86°; when dried at 78° and 0.02 mm. for 3 hrs. m.p.98-99°. When purified by sublimation at 140-145° (bath temp.) and 0.05 mm. the melting point was 100-101° whilst an authentic sample melted at 101-102°. The mixed melting point was not depressed. The ultraviolet absorption spectrum had the following characteristics:

λ max.	224	262.5	(270)	298
log ϵ max.	4.540	4.006	3.979	3.900

2-Carboxy-3-ethyl-1:2:3:4:6:6-hexahydro-9:10-dimethoxybenzo(a)-quinolizidine.

The amino ester (2.874 g.) was heated on the steam bath for 6 hrs. with 0.345N barium hydroxide (75 ml.) and then heated under reflux for 1½ hrs. The barium ion was precipitated by addition of 0.594N sulphuric acid to the cooled solution and the pH was adjusted to pH 5. The precipitate was digested for 2 hrs. on the water bath before filtering the solution. The filtrate on evaporation to dryness gave a partially crystalline amino acid (2.767 g.) which recrystallised from water (10 ml.) as shining white cubes (1.014 g.; m.p.197-199° sintering from 185°). The mother liquors were taken to dryness and the residue was redissolved in ethanol (5 ml.) when a second crop of crystals (450 mg.) was obtained; the mother liquors were reserved. A specimen for analysis was prepared by recrystallisation of the first crop from ethanol and was dried at 110° and 0.1 mm. over phosphoric oxide for 2½ hrs.

Analysis	C:63.4	H:8.0	N:3.7
$C_{18}H_{25}O_4N1H_2O$ requires	C:64.1	H:8.1	N:4.1

2-Carbomethoxy-3-ethyl-9:10-dimethoxybenzpyridocoline

The recrystallised amino acid (500 mg.) was esterified by heating under reflux with absolute methanol (50 ml.) and sulphuric acid (3 ml.) for 4 hrs. The ester (480 mg.) was

isolated as in the previous experiment and distilled at 130-140° (bath) and 0.05 mm. as a pale yellow gum.

Found	C:69.1	H:8.3	N:4.5
$C_{19}H_{27}O_4N$ requires	C:69.3	H:8.2	N:4.2

The ester crystallised in rosettes of needles and was recrystallised from 40-60° petroleum ether when its melting point was 56-57°.

$[\alpha]_D = +80.4°$ (C = 2.63 in ethanol)

Attempted epimerisation of amino ester

The ester (151 mg.) was dissolved in magnesium dried methanol (5 ml.) and a 0.1 M methanolic solution of sodium methoxide (1.2 ml.) was added. The solution was heated under reflux for 13 hrs., cooled, treated with 2N hydrochloric acid (0.5 ml.) and poured into saturated potassium carbonate solution (15 ml.). The liberated ester was extracted with ether (3 x 30 ml.) and then distilled at 130-140° (bath) and 0.05 mm. The distillate had $[\alpha]_D = +80.8°$ (C = 2.77 in ethanol).

Iso-nicotinic acid

γ-Picoline (31 g., 0.33 moles) was dissolved in water (200 ml.) and saturated sodium carbonate solution (2 ml.) added. The solution was heated on the steam bath and potassium permanganate (115 g., 3.3 equivs.) added over 4 hrs. with vigorous mechanical stirring. When the

permanganate colour had been destroyed the reaction mixture was filtered and the filter pad washed with water. Hydrochloric acid was added carefully to the filtrate to bring it to pH 5.0-5.2 when the iso-nicotinic acid (22.4 g., 53%) crystallised.

Methyl iso-nicotinate

Iso-nicotinic acid (22.4 g.) was heated under reflux with absolute methanol (100 ml.) and concentrated sulphuric acid (10 ml.) for 13 hrs. The volume of the solution was then concentrated to 25 ml. when it was poured into saturated potassium carbonate solution (200 ml.) and the ester extracted into ether (4 x 100 ml.) Evaporation of the ether gave methyl iso-nicotinate (14.4 g., 58%).

1-Methyl-4-carbomethoxy-piperidine

Methyl iso-nicotinate (14.4 g.;0.105 mole) was dissolved in ether (100 ml.) and heated under reflux for 2 hrs. with methyl iodide (37 ml.;0.25 mole) with a rigid exclusion of light. The bright red methiodide which crystallised overnight was collected and recrystallised from ethanol.

The methiodide was dissolved in water (50 ml.) and shaken with freshly precipitated silver chloride (28 g.). The silver salts were filtered off and the colourless filtrate hydrogenated in the presence of sodium acetate trihydrate (40.8 g.) and platinic oxide (500 mg.). The solution turned yellow and then colourless again as

reduction proceeded. Uptake of hydrogen ceased after 7.23 litres (0.303 mole) had been absorbed.

The solution was filtered to remove the catalyst, basified by addition of ammonia and extracted into ether (3 x 400 ml.). Evaporation of the ether gave the ester (10.8 g., 62%) which distilled at 86-87° under 14 mm.Hg. pressure. The base formed a picrate (recrystallised ethanol m.p.147-8°) and a perchlorate (recrystallised aqueous ethanol m.p.138-9°). The picrate analysed for C:43.6 H:4.6 N:13.6

$C_{14}H_{18}O_9N_4$ requires C:43.4 H:4.7 N:14.5

1-Methyl-4-hydroxymethyl-piperidine

A solution of 1-methyl-4-carbomethoxy-piperidine (1.01 g.; 6.4×10^{-3} mole) in dry ether (40 ml.) was added to a slurry of lithium aluminium hydride (500 mg.) in sodium dried ether (50 ml.) and stirred for 30 mins. at room temperature. Water (3 ml.) was added to decompose the excess lithium aluminium hydride and the ether solution filtered. Evaporation of the ether solution left 1-methyl-4-hydroxymethyl-piperidine (800 mg.; 99%). The base formed a picrate (recrystallised ethanol m.p.119-20°) which in admixture with the picrate of 1-methyl-4-carbomethoxy-piperidine depressed the melting point by 8°.

The picrate, recrystallised from ethanol, analysed for

C:43.6 H:5.3 N:15.7

$C_{13}H_{18}O_8N_4$ requires C:43.5 H:5.1 N:15.6

1-Methyl-4-chloromethyl-piperidine

1-methyl-4-hydroxymethyl-piperidine (1 g.) was dissolved in dry ether (20 ml.) and added dropwise to an ice-cold solution of thionyl chloride (10 ml.). When addition was complete the temperature was allowed to rise to room temperature whilst the solution was stirred vigorously. During this time the white crystalline hydrochloride separated out but when it proved to be extremely hygroscopic it was not collected. Instead the ether was evaporated and the thionyl chloride solution heated under reflux for ½ hr. after which the excess thionyl chloride was removed by distillation and the 1-methyl-4-chloromethyl-piperidine hydrochloride precipitated by addition of ether. The precipitate was collected and dried over phosphorus pentoxide in vacuo.

In a repeat experiment the base (1.02 g.) was recovered and converted into a picrate (m.p.193-4°) which analysed for

C:41.6 H:4.5 N:15.3

$C_{13}H_{17}O_7N_4Cl$ requires C:41.45 H:4.54 N:14.8

Attempted preparation of 1-methyl-4-cyanomethyl-piperidine

The hygroscopic 1-methyl-4-chloromethyl-piperidine hydrochloride (above) was dissolved in ethanol (20 ml.) and

water (10 ml.). Sodium cyanide (3 g.) was added and the solution heated under reflux for 11 hrs. Only a trace of material (4 mg.) was recovered into ether on basifying the solution whilst the aqueous layer remained a deep reddish-brown colour.

1-Methyl-4-hydroxymethyl-piperidinium benzochloride

1-Methyl-4-hydroxymethyl-piperidine (1.47 g.) was heated on a steam bath for 2 hrs. with benzyl chloride (10 ml.) when an oily layer separated which resinified upon cooling. This resin, which was extremely hygroscopic, was dissolved in methanol (8 ml.) and excess ether added to precipitate the quaternary material. This process was repeated with the precipitate and the product (3.0 g.) dried in vacuo.

Attempted conversion into 1-methyl-4-cyanomethyl-piperidine

1-methyl-4-chloromethyl-piperidinium benzochloride (1.5 g.) was dissolved in chloroform (50 ml.) and added dropwise to thionyl chloride (20 ml.). When addition was complete the solution was heated on the water bath for 2 hrs. after which the chloroform and excess thionyl chloride were removed by distillation. As the final traces of solvent were removed the material darkened and resinified. This resin was washed thoroughly with ether and dissolved in water when an insoluble tar (720 mg.) was rejected. Filtration of the aqueous solution (charcoal) gave a clear pale yellow filtrate to which sodium cyanide (1 g.) and three crystals of potassium iodide were added. After boiling for 16 hrs. under reflux

the solution was concentrated to low bulk under reduced pressure. The excess cyanide was then removed as hydrogen cyanide after acidification with hydrochloric acid.

The solution would not hydrogenate over a 10% palladium charcoal catalyst and the iodide ions were removed from the solution by shaking it with silver chloride (1 g.). The catalyst and silver salts were extracted with boiling ethanol after the solution had been filtered and the combined filtrates were concentrated to 70 ml. when they were hydrogenated over a palladium charcoal catalyst (300 mg.). The uptake of hydrogen was 44.5 ml.

The base (50 mg.) which was recovered from this hydrogenation formed a picrate (m.p.175-80°) which on recrystallisation melted at 192-3° and was identical with 1-methyl-4-chloromethyl-piperidine picrate.

The experiment was repeated using potassium cyanide and potassium iodide, and potassium cyanide (2 g.) and mercuric cyanide (12 mg.) at pH 5-6. No conversion to nitrile was obtained.

Barbier-Wieland Degradation of 1-methyl-4-carboxymethyl-piperidine

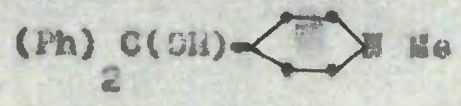
A solution of phenyl magnesium bromide was prepared by reacting magnesium (2.68 g.) with bromobenzene (17.9 g.) in dry ether (30 ml.). 1-methyl-4-carboxymethyl-piperidine (1.01 g.) was then dissolved in dry ether (10 ml.) and added

to the solution of the Grignard reagent over a period of 3 mins. with rapid stirring. The solution was then heated under reflux for 4½ hours when it was cooled and poured on to a mixture of crushed ice (40 g.) and concentrated hydrochloric acid (7 ml.). The crude hydrochloride (2.1 g.) was collected and washed with water, ethanol and ether. When it was recrystallised from water the hydrochloride had a melting point 291-2°. The base crystallised from 40-60° petroleum ether in needles (m.p.132-3°) which analysed for

	C:80.8	H:8.2	N:5.0
$C_{19}H_{23}ON$ requires	C:81.1	H:8.3	N:5.0

The ultraviolet absorption of the hydrochloride in aqueous solution exhibited a minimum at 246 mu ($\log \epsilon = 2.615$) and a maximum at 258 mu ($\log \epsilon = 2.808$).

Dehydration of 1:1-diphenyl-N-methyl-4-piperidyl carbinol



(a) With acetic acid - acetic anhydride

The base (460 mg.) was heated under reflux for 1 hour with acetic acid (20 ml.) and acetic anhydride (10 ml.). After a further 3 hours at room temperature the recovered base had a m.p.125-7° which was raised to 131-3° in admixture with the starting material. The base formed an extremely insoluble hydrochloride and was not hydrogenated over a platinum catalyst.

(b) With a 40% solution of Boron trifluoride in acetic acid

The base (1.59 g.) was dissolved in glacial acetic acid (25 ml.) and the boron trifluoride reagent (5 ml.) added. This solution was heated under reflux for 1 hr. after which acetic acid (10 ml.) was removed by distillation. Upon addition of water (20 ml.) a white crystalline material (1.13 g.) was precipitated and collected. It was insoluble in ether, ethanol and water and was stable to sodium hydroxide solution. The material had a crude melting point of 208-9° and was presumably a boron trifluoride complex with the carbinol.

From the filtrate was recovered a base (500 mg.; m.p. 51-2°) which gave a crystalline perchlorate (recrystallised ethanol m.p. 239-40°). The base could not be recrystallised from ether, petroleum ether, ethanol, methanol or aqueous ethanol. The boron trifluoride complex dissolved in a hot mixture of acetic acid (8 ml.) and water (2 ml.). When this solution was neutralised with sodium hydroxide solution a base (839 mg.) was extracted into ether. The base formed a perchlorate (m.p. 239-40°) which was identical with that obtained from the filtrate and analysed for C:62.2 H:6.1 N:3.9

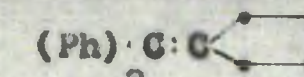
$C_{19}H_{22}O_4NCl$ requires C:62.7 H:6.1 N:3.85

The ultraviolet absorption curve of the perchlorate was very flat but possessed a maximum at 243 ($\log\epsilon = 4.180$).

Both salt and free base were resistant to catalytic

hydrogenation.

Oxidation of 1,1-diphenyl-N-methyl-4-piperidylidene methane



(4-Benzhydrylidene-N-methyl-piperidine)

The base (142 mg.) was dissolved in acetone (20 ml.) and a solution of potassium permanganate (128 mg.) in aqueous phosphate buffer of pH 6.4 was added dropwise with stirring. After heating under reflux for 2 hours the colour of the permanganate had been destroyed. The manganese dioxide was removed by filtration. The filtrate was combined with the acetone extracts of the filter pad and evaporated to low bulk. This aqueous concentrate was extracted with ether to yield a neutral fraction (63 mg.) and a basic fraction (39 mg.).

The neutral compound formed a dinitrophenylhydrazone (26 mg., m.p.238-9°). Benzophenone dinitrophenylhydrazone (m.p.238-9°) did not depress the melting point in admixture.

The base formed a perchlorate (m.p.213-5°). However, the yields of both base and benzophenone were low.

(b) Ozonolysis

An attempt to achieve fission of the molecule by ozonolysis was unsuccessful.

Rubremetaminium Bromide

Emetamine (139 mg.) recovered from the hydrogen oxalate and potassium acetate (50 mg.) were dissolved in water (8 ml.) and glacial acetic acid (0.4 ml.). Mercuric acetate (850 mg.) was added and the solution was heated under reflux for 6½ hours, then cooled and filtered free from the precipitated mercurous acetate. The filter pad was washed successively with water, hot acetone (100 ml.) and hot ethanol (100 ml.). The combined filtrate and washings, concentrated to remove the alcohol and acetone, were treated at the boiling point with hydrogen sulphide for 10 minutes. The solution was then acidified with dilute hydrochloric acid and hydrogen sulphide was passed for a further 5 minutes. The precipitated mercuric sulphide was collected and extracted with boiling ethanol (300 ml.). The extract was concentrated and added to the main filtrate which was evaporated to dryness under reduced pressure. The deep red crystalline chloride was dissolved in boiling water (30 ml.) and 48% hydrobromic acid (2 drops) added. The rubremetaminium bromide (112 mg., 58%) was recrystallised from water (10 ml.) and dried in a vacuum dessicator for 24 hours when it contained water of crystallisation (15%) [$C_{29}H_{31}O_4N_2Br.6H_2O$ requires 16%]. Rubremetaminium bromide sinters 195-225°. U.V. Absorption spectrum in water : max. 257, 306, 330, 355, 375, 460 mu (max. 72,610; 16,870; 12,160; 12,110; 12,360; 19,360): min. 280, 321, 340, 360, 403, (min. 11,690; 11,030; 9,705; 11,970; 7,240) - see fig.10, appendix.

Oxidation of Emetamine Hydrogen Oxalate and Isolation of a Further Oxidation Product.

To a solution of emetamine hydrogen oxalate (392 mg.) and sodium acetate (100 mg.) in water (5 ml.) and acetic acid (0.8 ml.) was added mercuric acetate (1.7 g.). The solution was heated under reflux for 25 minutes after which the precipitated salts were filtered off and extracted with water and ethanol. The washings were concentrated to remove the alcohol and combined with the main clear red filtrate. Mercuric acetate (700 mg.) was added to this solution and heating was continued for 17 hours. The mercurous acetate was then filtered off and excess mercuric and mercurous ions were precipitated in the usual way as the sulphide . Evaporation of the solution so obtained under reduced pressure left a bright red crystalline solid. This dissolved readily in warm acetone (1 ml.) and water (10 ml.) and 48% hydrobromic acid (3 drops) was added. After the acetone had been boiled off orange-red crystals were obtained. They were converted back into the acetate by suspending the bromide in hot water (15 ml.) and shaking with an excess of silver acetate. The silver salts were filtered off and washed well with acetone. The aqueous acetone solution was concentrated to 15 ml. when the red acetate (171 mg.) was collected and dried in a dessicator. The mother liquor was heated under reflux for 12 hours with mercuric acetate (500 mg.) but yielded only a dark brown resinous material which was not

further examined. The acetate was dissolved in the minimum of boiling water and 40% hydrobromic acid added. The quaternarybromide was collected and recrystallised from acetone and dilute hydrobromic acid (15 ml.). Rubremetaminium bromide crystallised out almost at once but overnight a crop of fine yellow needles separated on top of the deep red rubremetaminium salt. The solution was warmed to dissolve the yellow crystals and the rubremetaminium bromide (63.3 mg.) was collected. The yellow needles (43 mg.) which crystallised from the filtrate were suspended in water, the suspension was basified with sodium hydroxide and thoroughly extracted with ether. From this extract no basic fraction was obtained. The aqueous suspension was reacidified by addition of dilute hydrobromic acid, warmed and the minimum of acetone added to dissolve the needles. On cooling, fine yellow needles (30 mg.; sint.170-175°) slowly crystallised. When these were recrystallised from dilute hydrochloric acid the sintering and decomposition occurred at 249°C. The U.V. absorption spectrum of the purified needles when determined in ethanolic solution was quite different from that of rubremetaminium bromide λmax. 257.5, (300), 311, 355, 381.5, 447 (log. O.D. max. 1.787, 1.704, 1.857, 1.533, 1.306). (fig.10)

ϵ max. 311: = 49,600 Calculated on M.wt. = 551, $C_{29}H_{31}O_4N_2Br$

" " = 52,200 " " " = 631, $C_{29}H_{31}O_4N_2Br_2$

Oxidation of Rubremetaminium Bromide with Mercuric Acetate

Rubremetaminium bromide hexahydrate (11 mg.) and sodium acetate (10 mg.) were dissolved in water (2 ml.) and glacial acetic acid (0.5 ml.); mercuric acetate (64 mg.) was added and the solution heated under reflux for 18 hours after which it was filtered to remove mercurous acetate. The boiling filtrate was saturated with hydrogen sulphide and then filtered free of the precipitated sulphides. The combined filtrate and ethanolic washings were concentrated to 1 ml. and acetone (10 ml.) was added when inorganic salts were again precipitated. This solution was filtered and the filtrate concentrated to 2 ml. Overnight scarlet-red crystals of rubremetinium bromide (1-2 mg.) and several bunches of golden yellow needles deposited. The needles in suspension in the mother liquor were separated from the rubremetaminium bromide by decantation and the suspension filtered to give golden yellow needles which were further purified by washing with ethanol. On addition of water (0.25 ml.) and 43% hydrobromic acid (0.25 ml.) to the mother liquor more needles crystallised. The combined crops (16 mg.) were recrystallised from dilute hydrobromic acid. The U.V. absorption spectrum of the purified needles when determined in ethanolic solution was identical with that of the yellow needles isolated in the previous experiment.

Microhydrogenation of Rubremetaminium Bromide

Rubremetaminium bromide (28-30 mg.) in ethanol (10 ml.) was hydrogenated in the presence of sodium acetate (33.5 mg.) and platinic oxide (20 mg.). Uptake of hydrogen (0.98 ml.; 1.02 mole; 18°C; 760 mm.) ceased after 30 minutes when the solution was a pale yellow colour and exhibited a strong green fluorescence. The solution was rapidly filtered and the catalyst washed with ethanol, water (10 ml.) and dilute hydrochloric acid (0.5 ml.). The combined washings and filtrate were concentrated to 8 ml. The aqueous solution was extracted with ether (10 ml.), then made alkaline with sodium hydroxide and extracted with ether (2 x 5- ml.). The colourless dried extract was evaporated to give a nearly colourless base (17 mg.) which having been left overnight in a stoppered flask with ether (1 ml.) crystallised in two forms. α - dihydrorubremetamine needles (m.p. 127-131°), β - dihydrorubremetamine buttons (m.p. 175-177°), which were separated manually. Recrystallisation of the needles from ether raised the melting point to 133-4° whilst the melting point of the buttons was raised to 205-6° on recrystallisation from ether. Purer samples prepared subsequently by Dr. Battersby analysed correctly for dihydrorubremetamine, melted at 143-145° and 219-221° respectively.

The U.V. absorption spectrum for the α- and β- dihydrorubremetamine were identical and showed λmax. 236.5; 264; (315); 328; (355) [εmax. 33,570; 34,580; 9,550; 11,300; 4,677] ; λmin. 247; 301.5 [εmin. 30,550; 6,887] (fig.11)

In a repeat hydrogenation experiment with rubremetaminium bromide (155 mg.) the aqueous layer was acidified with dilute hydrobromic acid after the extraction with ether and concentrated to 5 ml. On addition of acetone (1 ml.) a small amount of material crystallised in fine yellow needles which had an identical U.V. spectrum to that of the yellow needles obtained by oxidation of rubremetaminium bromide. The yellow needles would thus appear to be resistant to hydrogenation and hydrogenation followed by extraction of the dihydrorubremetamine would appear to afford a simple way of purifying the yellow needles from contaminating rubremetaminium bromide.

Reoxidation of α- Dihydrorubremetamine

α- dihydrorubremetamine (0.29 mg.) in ethanol (25 ml.) was heated on the steam bath for 1 hour with mercuric acetate (10 mg.) and acetic acid (0.2 ml.). Yellow colour developed at once. After 1 hour the solution was filtered, evaporated to dryness and redissolved in water (15 ml.). Hydrogen sulphide was passed through the solution, dilute hydrochloric acid then added and hydrogen sulphide passed again. No precipitate was obtained. The solution was filtered clear and submitted for U.V. analysis when the spectrum (graph12) indicated that the majority of the material had been re-oxidised beyond rubremetaminium bromide.

Rubremetinium Bromide

Emetine hydrobromide (6.0g.) and sodium acetate (2.g.) were dissolved in water (40 m.p.) and acetic acid (10 ml.). Mercuric acetate (19.9 g.) was added and the solution heated under reflux for 8 hours at 130° (bath). On working up as for rubremetaminium bromide, rubremetinium bromide (2.94 g., 64%) was obtained.

Oxidation of Rubremetinium Bromide with Mercuric Acetate

Rubremetinium bromide (384 mg.) was dissolved in water (5 ml.) with acetic acid (2 ml.). Mercuric acetate (1 g.) was added and the solution heated under reflux for 20 hours at 148° (bath temp.). No mercurous acetate separated but several small globules of mercury were formed. Hydrogen sulphide was passed through the boiling solution, the mercuric sulphide coagulated and hydrogen sulphide was passed again. The solution was filtered and the filterpad extracted with boiling ethanol. Evaporation of the combined filtrates to dryness left orange-red crystals which were suspended in water (8 ml.) then collected and washed with water. The air-dried crystals were then dissolved in ethanol (25 ml.) and hydrogenated in the presence of platinic oxide (150 mg.). After 1½ hours uptake of hydrogen (42 ml.) had ceased and the original red colour had given way to a pale yellow one exhibiting an intense green fluorescence. The solution was filtered under nitrogen, the filtrate concentrated to 10 ml. then made

alkaline with sodium hydroxide and extracted with ether (2 x 20 ml.). The aqueous layer was acidified with acetic acid and concentrated to 3 ml. Treatment with dilute hydrobromic acid gave crystalline material (16 mg.) which was recrystallised from dilute hydrobromic acid and submitted for U.V. analysis. U.V. spectrum max. 258.5; 309; 311; 384; 449; min. 236.5; 292; 302; 331.5; 433. (fig.9).

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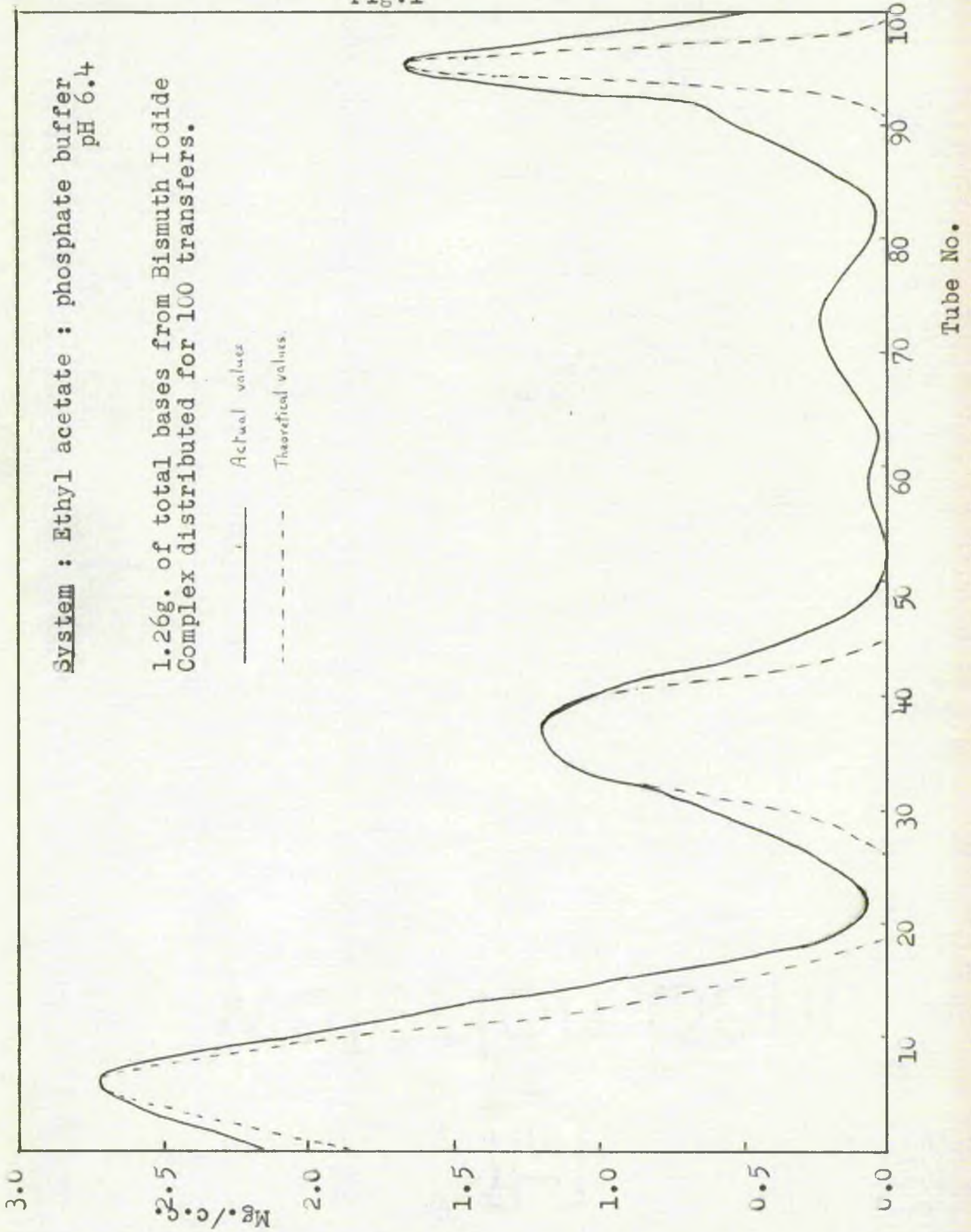
63.	Merchant & Marvel	J.A.C.S.	1928	50, 119
64.	Jones, Davy & Halsall	J.C.S.	1951	269
65.	Greenwood & Martin	Quart. Revs.	1954	8, 1
66.	Braude & Coles	J.C.S.	1950	2014
67.	Wood	Ph.D. Thesis (St. Andrews University)		
68.	Wiesner, Clarke & Kairys	Can. J. Research	1950	234

69.	Ipatiev	Ind. Eng. Chem.	1936	28, 22
70.	Bockelheide & Godfrey	J.A.C.S.	1953,	75, 36
71.	Openshaw	The Chemical Society: Special publication No. 3	1955	p.2
72.	Leonard			
73.	Feller & Forschirmer	Monatsh	1949	80, 94
74.	Battersby & Openshaw	Experientia	1949	5, 398
75.	Campbell, Tipson & Elderfield)	Proc.Roy.Soc.	1938B	125, 6
76.	Harding, Haworth & Perkin	J.C.S.	1906	93, 19
77.	Lyle and Lyle	J.A.C.S.	1954	76, 353
78.	Speth & Polgar	Monatsh		51, 19
79.	Decker & Klauser	Ber.	1904	37, 5
80.	Battersby, Davidson & Harper	J.C.S.	1959,	1740
	Battersby & Harper	J.C.S.	1959,	1748
	Battersby, Binks & Davidson	J.C.S.	1959,	2704
	Battersby & Garratt	J.C.S.	1959,	3512
	Battersby & Turner	J.C.S.	1960,	717
	Battersby, Binks & Edwards	J.C.S.	1960,	3474
	Battersby, Davidson & Turner	J.C.S.	1961,	3899

Appendix.

Ultraviolet absorption curves and counter-current distribution curves.

Fig.1


System : Ethyl acetate : phosphate buffer pH 6.4
1.26g. of total bases from Bismuth Iodide Complex distributed for 100 transfers.
Actual values
Theoretical values
Mg./c.c.
3.0
2.5
2.0
1.5
1.0
0.5
0.0
10
20
30
40
50
60
70
80
90
100
Tube No.

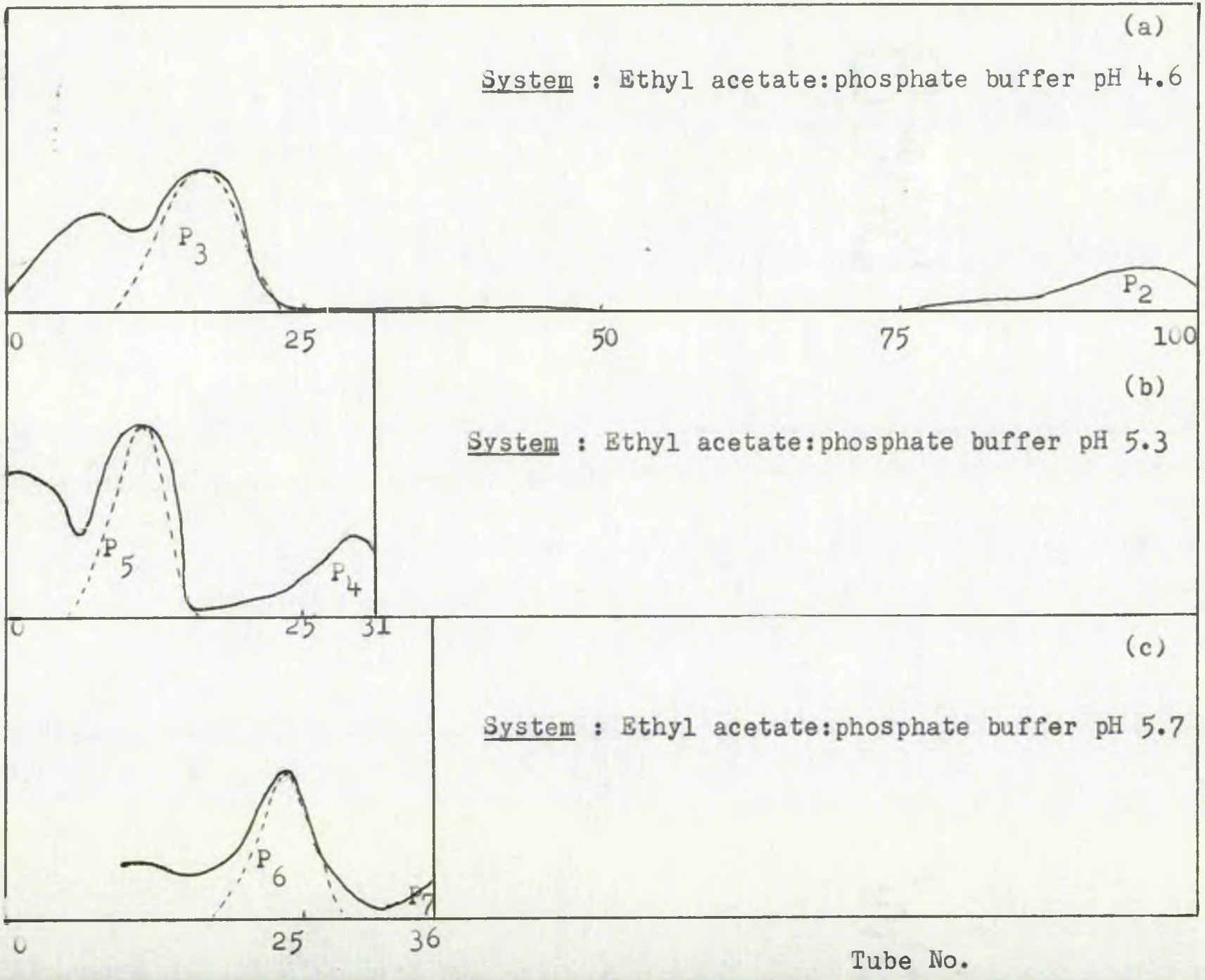

(a)
System : Ethyl acetate:phosphate buffer pH 4.6
P3
P2
0
25
50
75
100
(b)
System : Ethyl acetate:phosphate buffer pH 5.3
P5
P4
0
25
31
(c)
System : Ethyl acetate:phosphate buffer pH 5.7
P6
P7
0
25
36
Tube No.

Fig.2

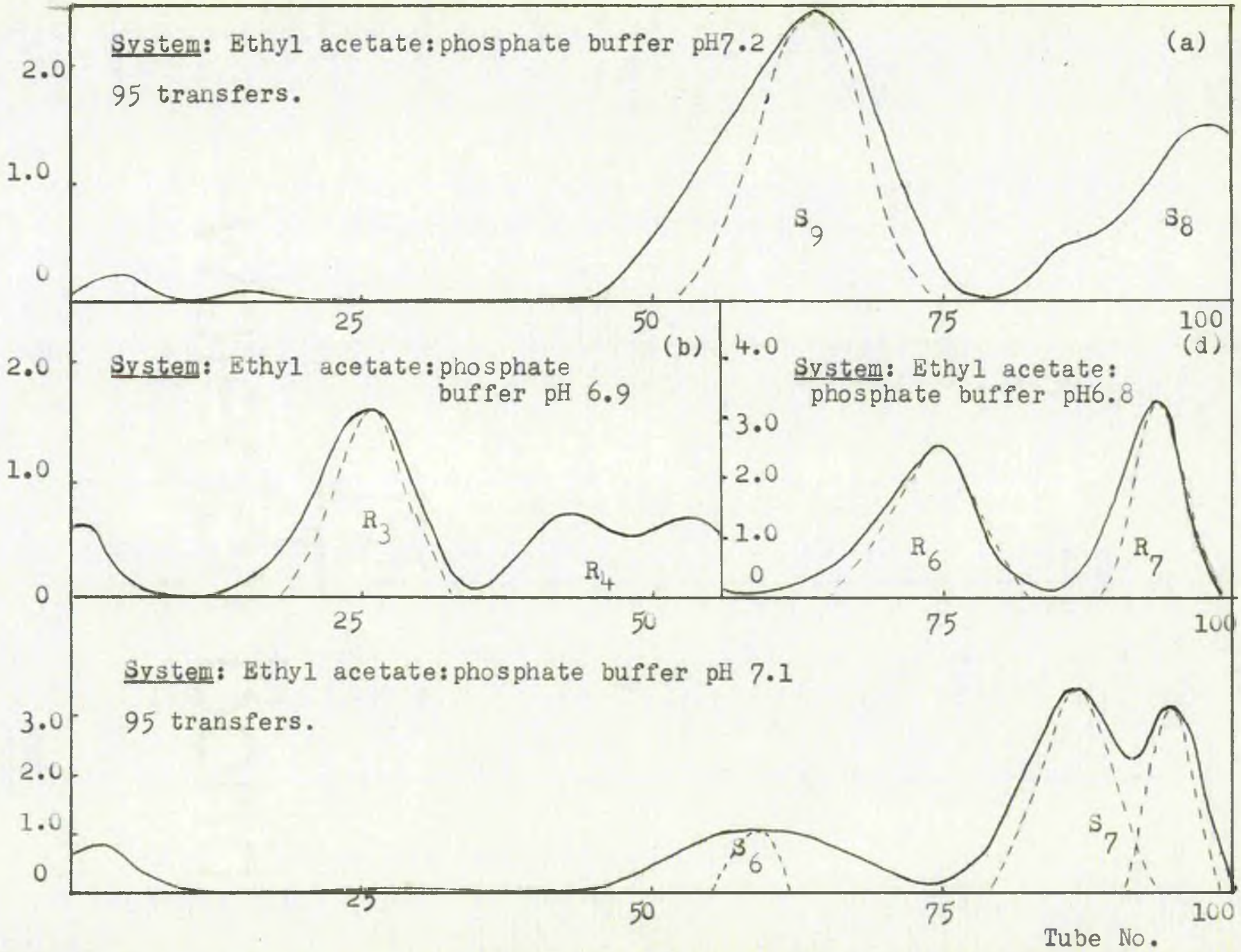

System: Ethyl acetate:phosphate buffer pH7.2
95 transfers.
(a)
2.0
1.0
0
S_9
S_8
25
50
75
100
(b)
System: Ethyl acetate:phosphate buffer pH 6.9
2.0
1.0
0
R_3
R_4
25
50
(d)
System: Ethyl acetate: phosphate buffer pH6.8
4.0
3.0
2.0
1.0
0
R_6
R_7
75
100
System: Ethyl acetate:phosphate buffer pH 7.1
95 transfers.
3.0
2.0
1.0
0
S_6
S_7
25
50
75
100
Tube No.

Fig.3

Fig. 4

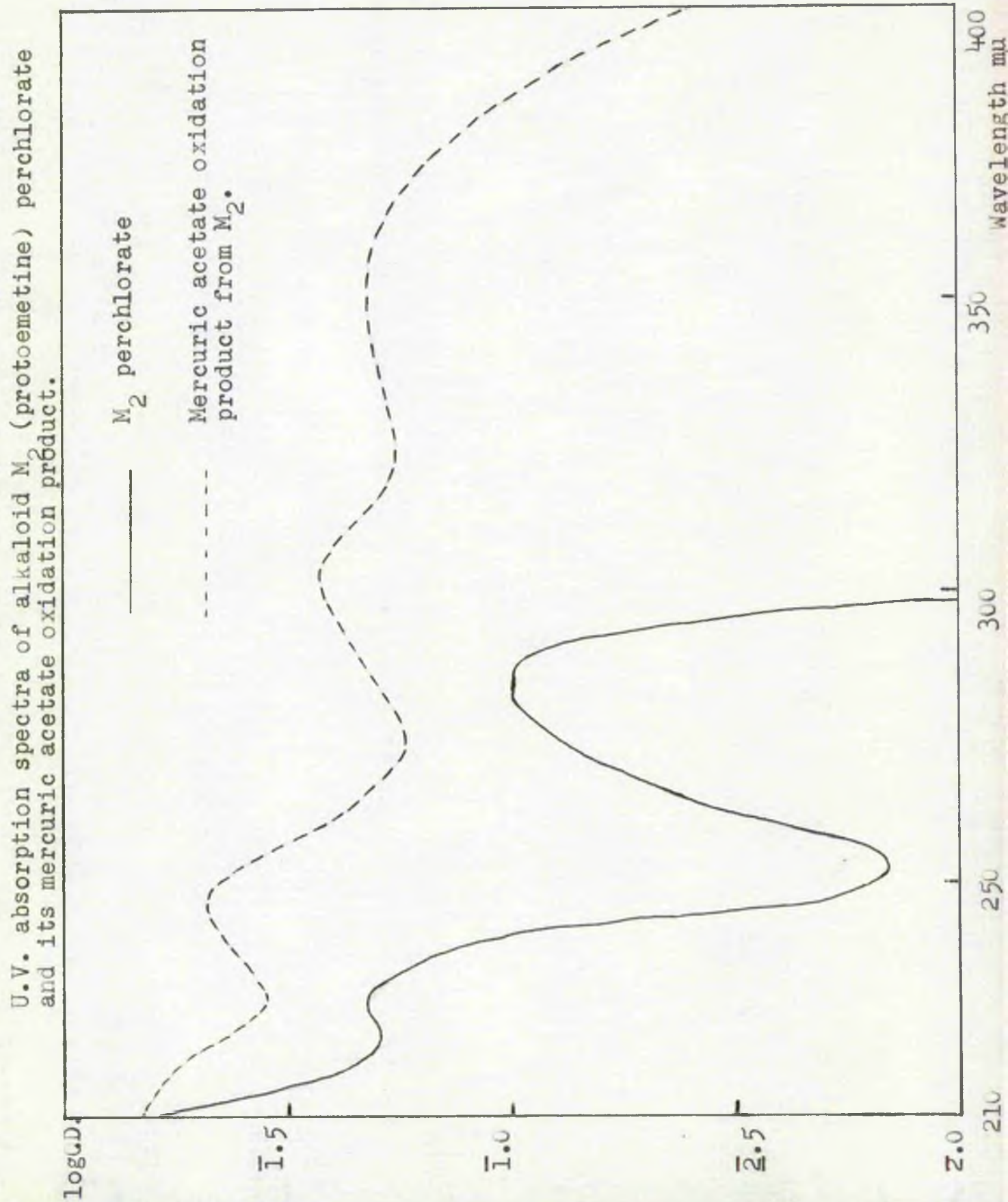

U.V. absorption spectra of alkaloid M_2 (protoemetine) perchlorate and its mercuric acetate oxidation product.
M_2 perchlorate
Mercuric acetate oxidation product from M_2.
log D
1.5
1.0
2.5
2.0
210
250
300
350
400
Wavelength mμ

Fig. 5

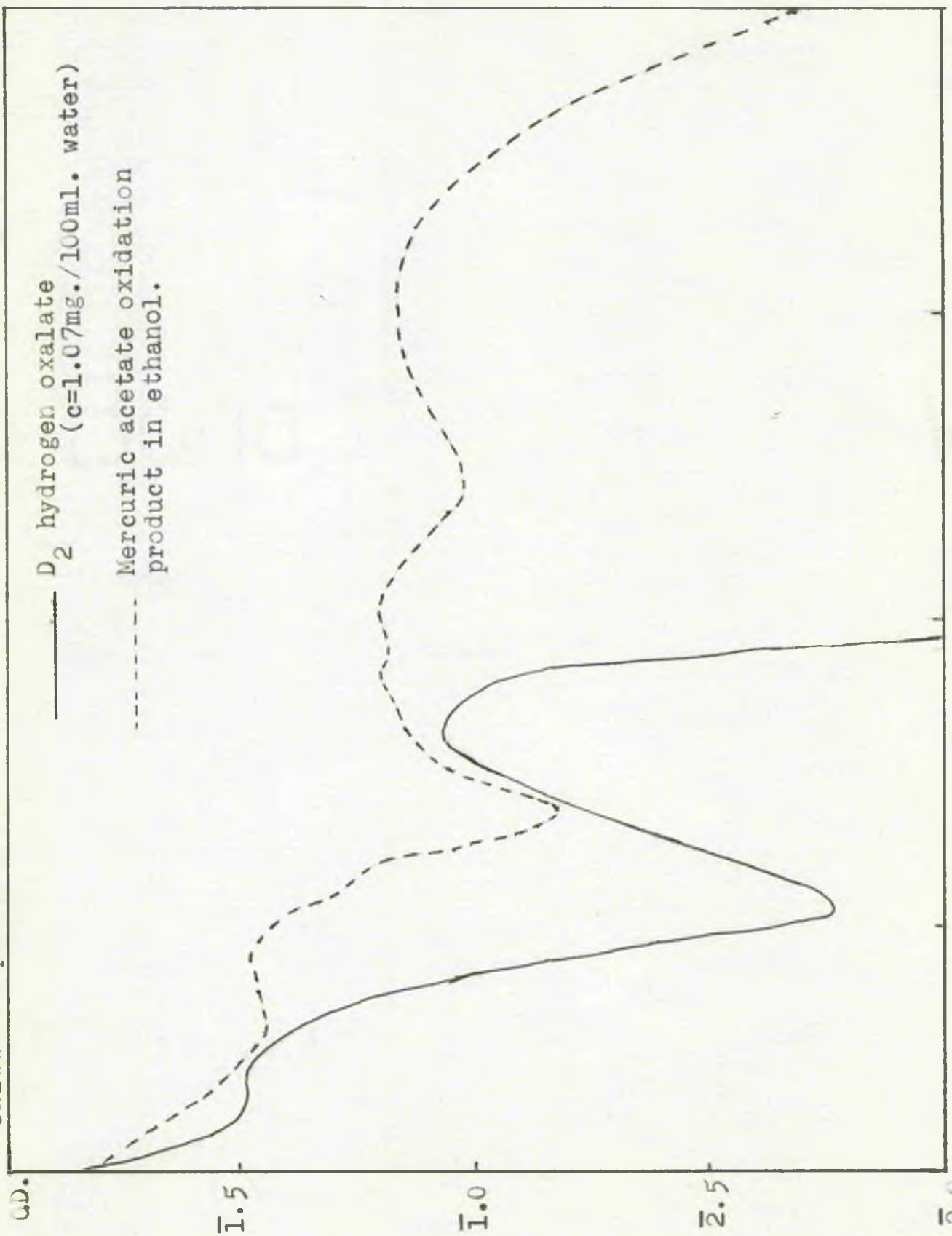

U.V. absorption spectra of D_2 hydrogen oxalate and its mercuric acetate oxidation product.
log O.D.
1.5
1.0
2.5
2.0
210
250
300
350
400
Wavelength mμ
D_2 hydrogen oxalate (c=1.07mg./100ml. water)
Mercuric acetate oxidation product in ethanol.

Fig.6 U.V. absorption spectrum of 1-keto-2-benzyl-6,7-dimethoxy-1,2-dihydroisoquinoline in ethanol.

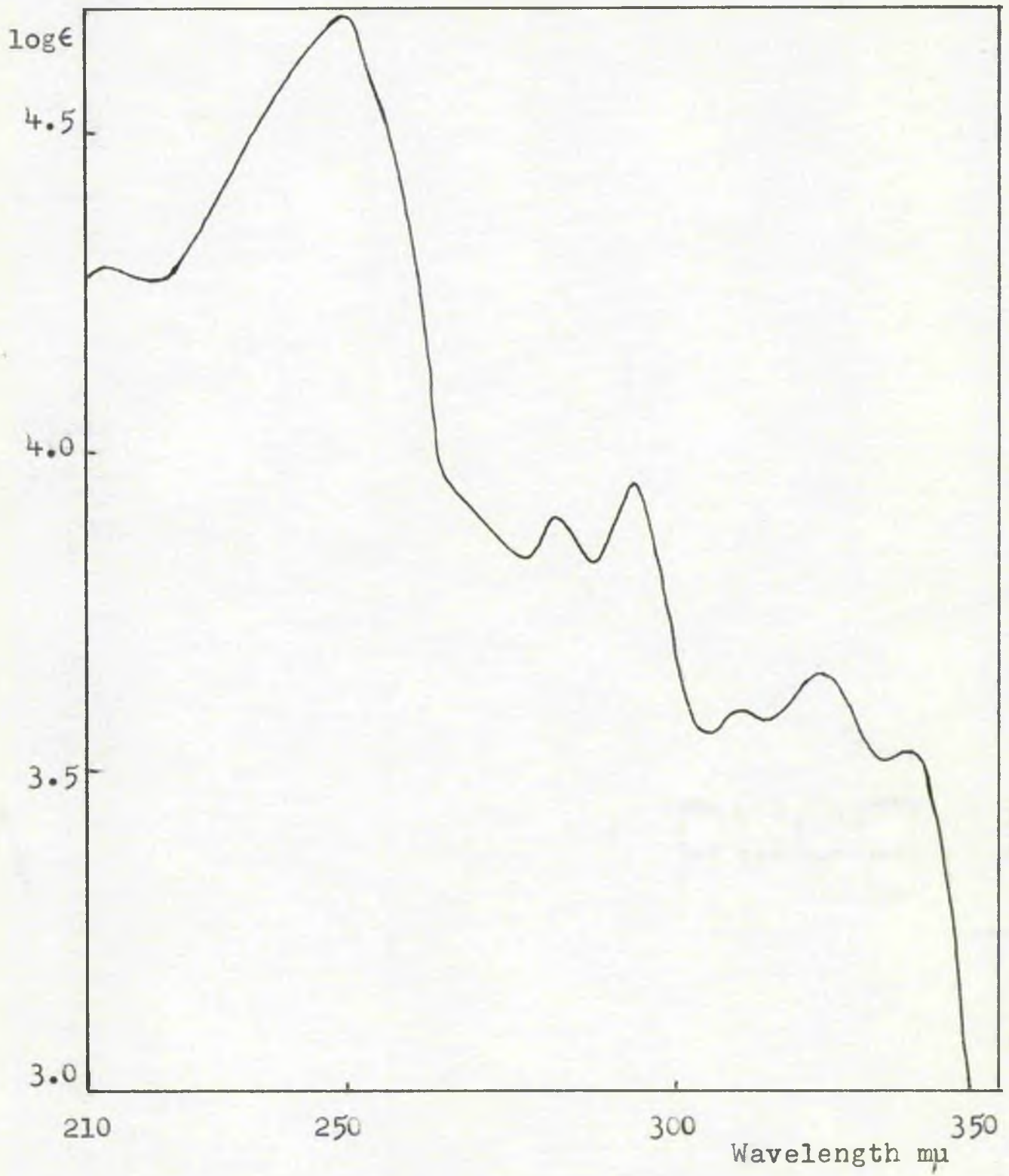


Fig.7

U.V. absorption spectrum of 1-keto-2-benzyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline in ethanol.

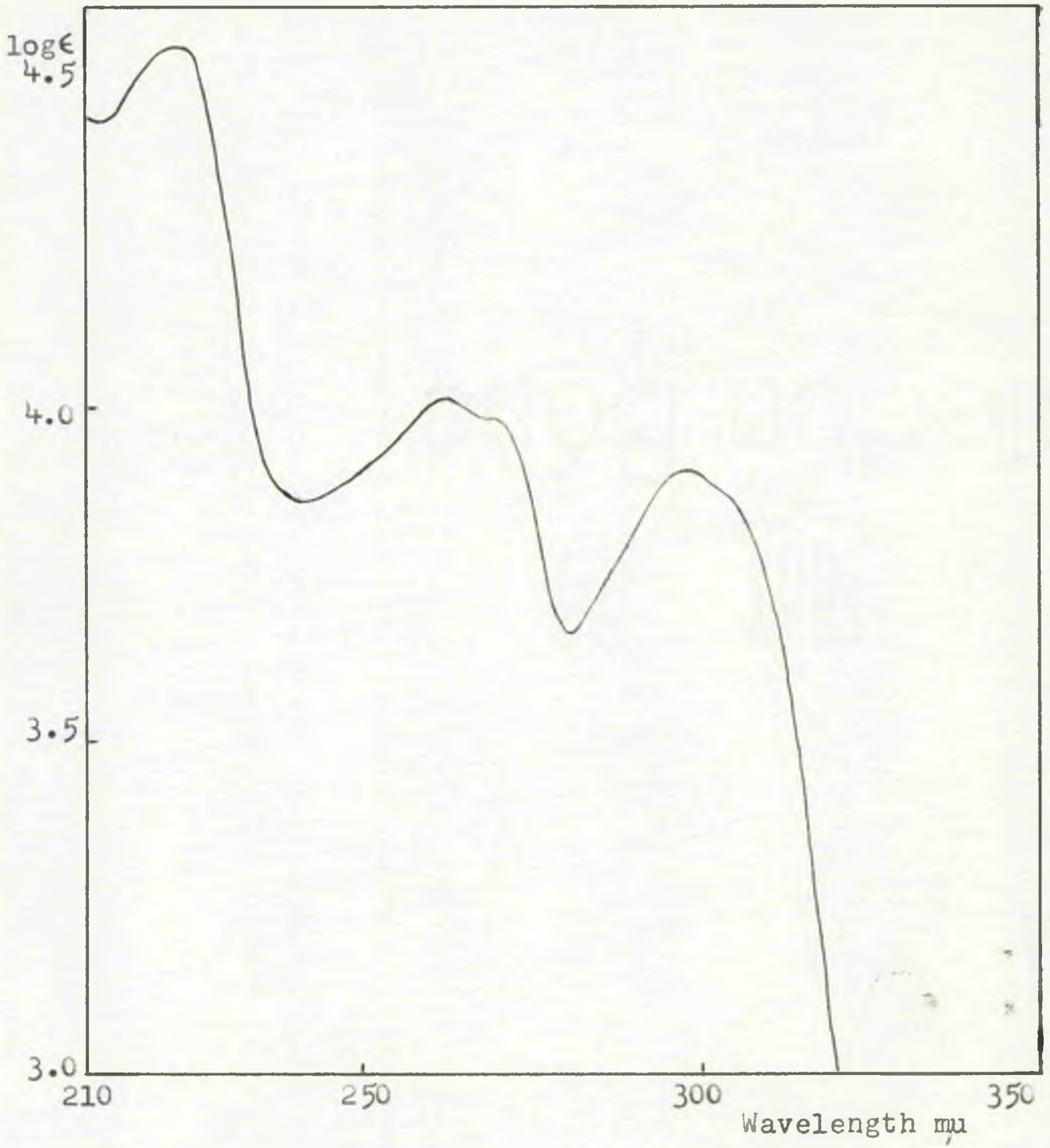


Fig.8

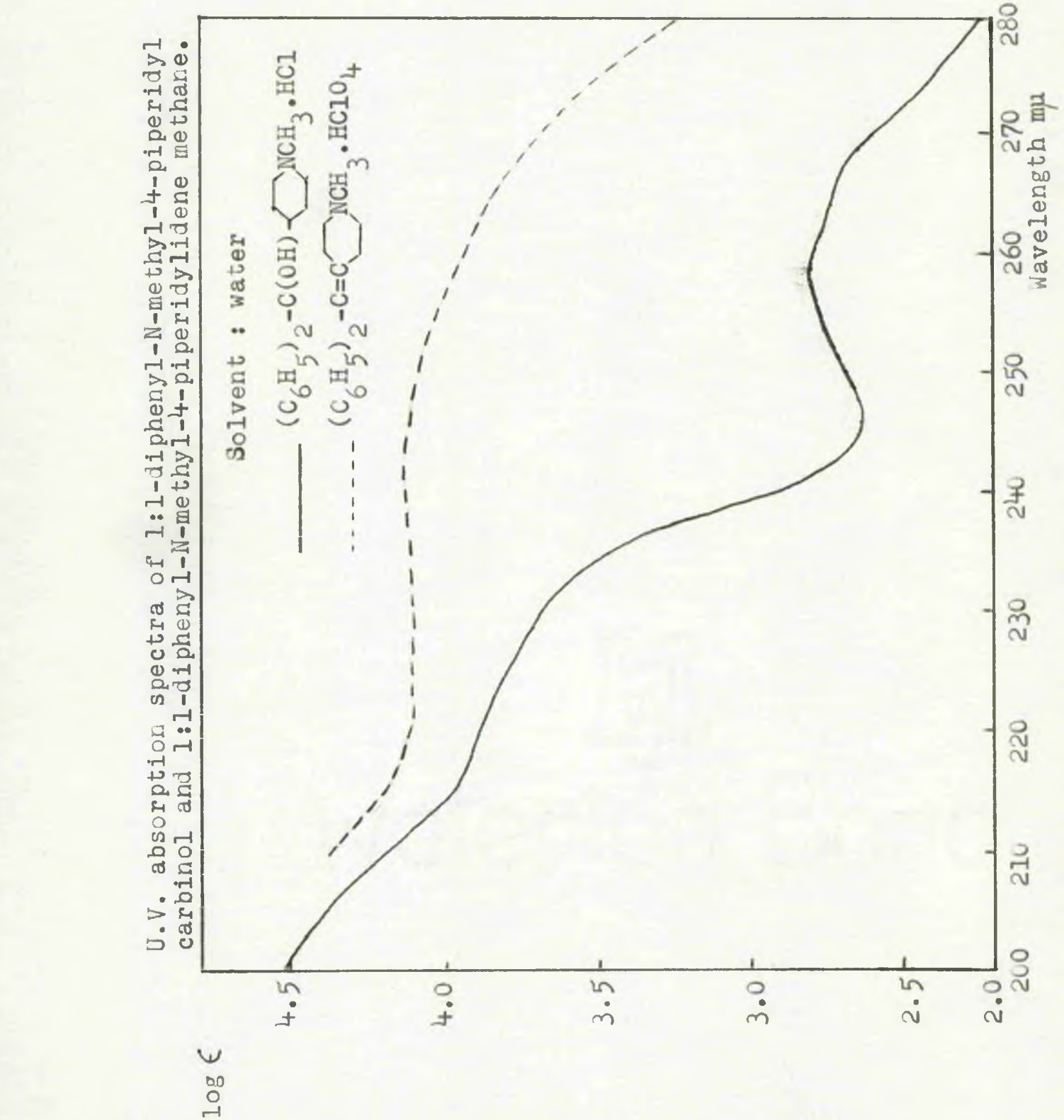
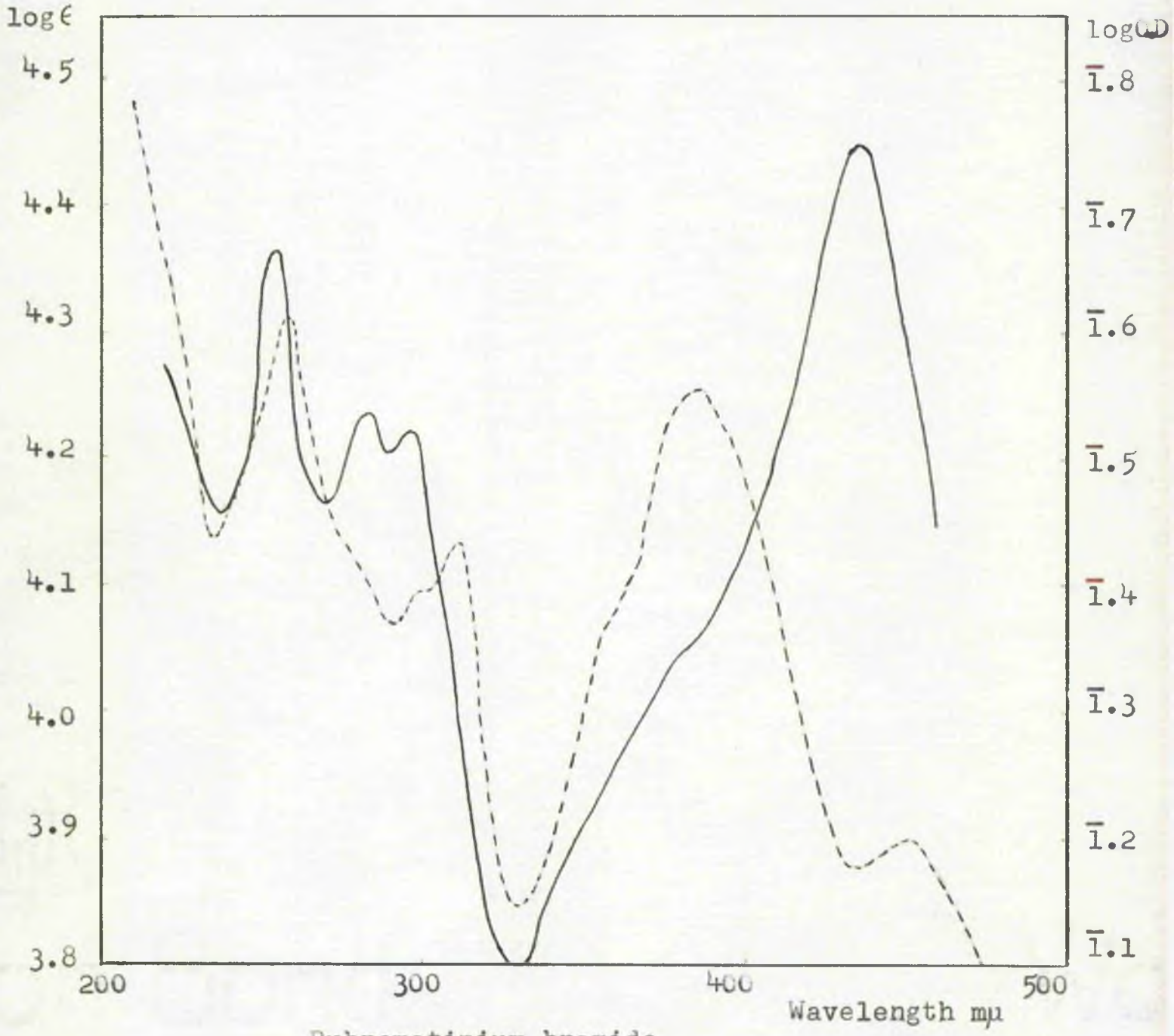

U.V. absorption spectra of 1:1-diphenyl-N-methyl-4-piperidyl carbinol and 1:1-diphenyl-N-methyl-4-piperidylidene methane.
Solvent : water
$(C_6H_5)_2$-C(OH)-NCH_3.HCl
$(C_6H_5)_2$-C=C-NCH_3.$HClO_4$
log ε
4.5
4.0
3.5
3.0
2.5
2.0
200
210
220
230
240
250
260
270
280
Wavelength mμ

Fig.9

Ultraviolet absorption spectra of rubremetinium bromide and its mercuric acetate oxidation product.

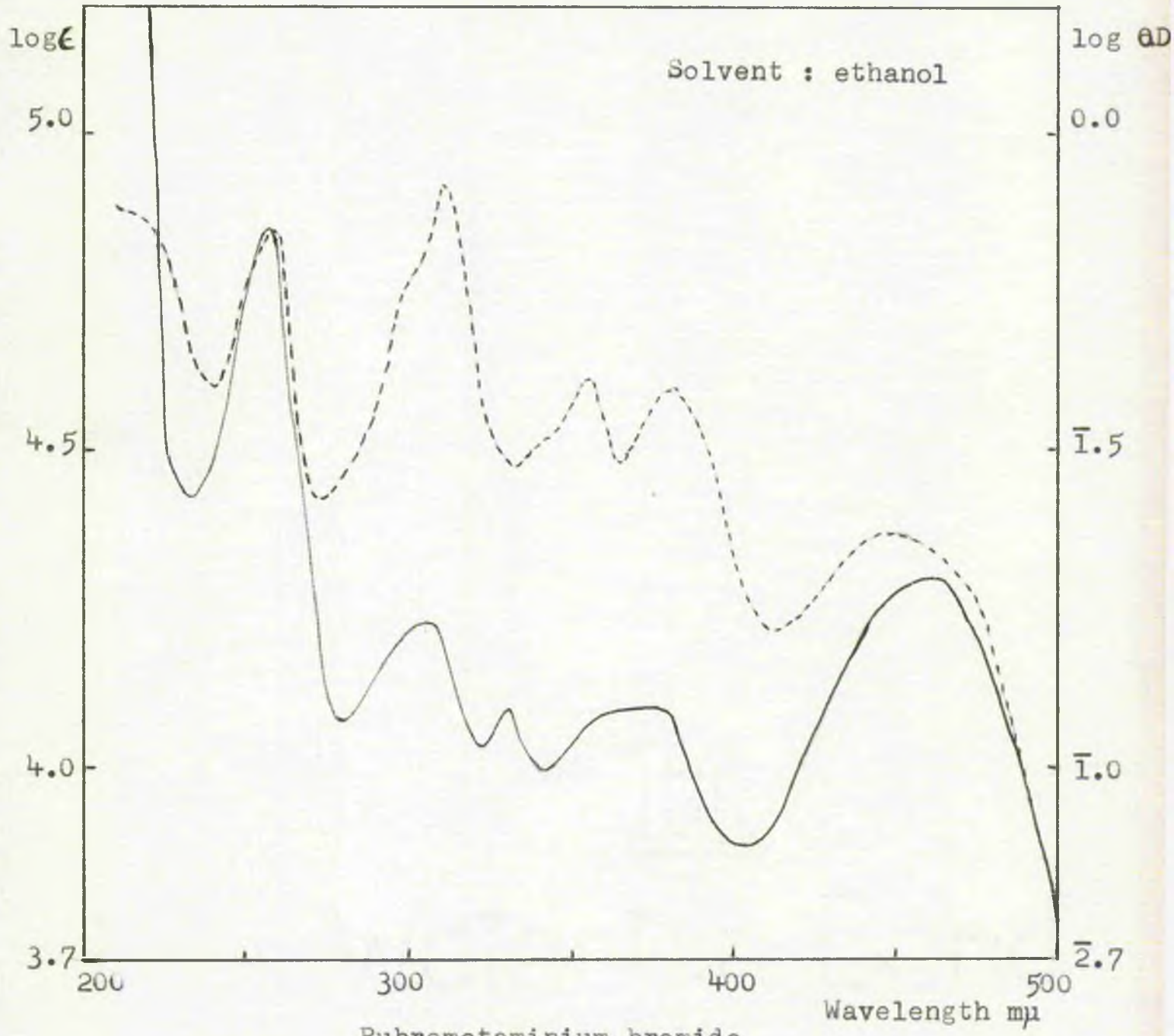


Rubremetinium bromide

Mercuric acetate oxidation product of rubremetinium bromide.

Fig.10

Ultraviolet absorption spectra of rubremetaminium bromide and its mercuric acetate oxidation product.



_____ Rubremetaminium bromide

- - - - - - Mercuric acetate oxidation product of rubremetaminium bromide.

Fig.11

U.V.absorption spectra of α- and β-dihydrorubremetamine and 3-methyl-7,8-benzopyrrocoline.

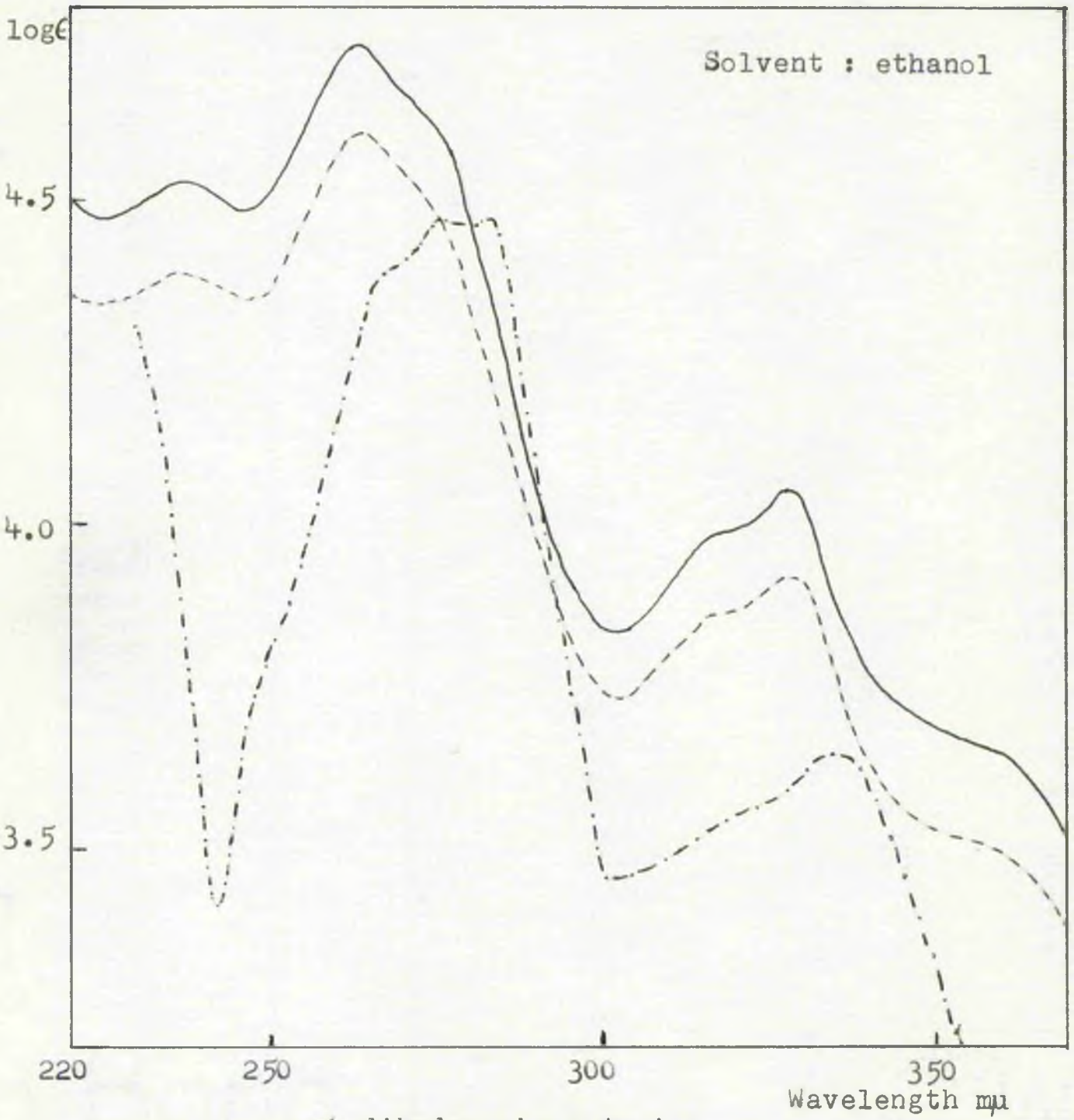


Fig.12

U.V.Absorption spectrum of the product obtained from the re-oxidation of dihydrorubremetamine with mercuric acetate.